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Platelet-rich plasma enhances regeneration of the affected skin in case of experimental dermatitis

V.N. Zaporozhan[1], E.L. Kholodkova[1], N.V. Neskoromnaya[1], I.V.Badyin[2]

1 - Odessa national medical university, Odessa, Ukraine

2 – Research institute of curortology and medical rehabilitation, Odessa, Ukraine

Keywords: skin; platelet-rich plasma; experiment; dermatitis; regeneration.

Summary

Dermatitis is an urgent problem in modern medicine. At the same time, classic treatment of the deep dermatitis with the affection of the hair follicles is not efficient. Recently, great attention is paid to the use of platelet-rich plasma (PRP) which is effective in ulcerative lesions of the lower extremities treatment, healing of postoperative wounds etc.

Therefore, the aim of the research was to study the impact of PRP on the regeneration of the skin of mice after induced contact dermatitis. The conducted studies have shown PRP promotes reparative regeneration of the skin, thus preserving the morphological properties of tissues and organ in general. This is provided with a number of growth factors that are released from platelets, enhancing proliferation of cellular elements in the affected area, as well as participate in the process of adaptation.

Latest decades are characterized by progressive growth of skin diseases what characterized by dystrophic affection and hair loss even after the treatment [3, 8]. Platelet-rich plasma (PRP) is promising for the different tissues regeneration [6, 7]. PRP is a small amount of blood plasma were the concentration of platelets riches 10x10 5 / ml and more. Platelets regulate new blood vessel growth through numerous stimulators and inhibitors of angiogenesis by several pathways, including differential exocytosis of angiogenesis regulators [1]. It was shown PRP positively influences on the ulcerative lesions of the lower limbs proving their healing [2], potencies fat grafting [9], and decreases pain after sport injuries [5].

Therefore, the aim of the research was to study the impact of PRP on the regeneration of the skin of mice after induced contact dermatitis.

Materials and Methods

Sixty adult female ICR mice, weighing 23-29 g were utilized in this study. Mice were fed a standard mice chow and tap water ad libitum. Temperature 22±2Cº and humidity 50-55 % were performed. Animals received human care in accordance with the European Communities Council Directive of November 24 1986 (86/609/EEC).

Experimental design

The mice were randomly allotted into two experimental groups: 1st with induced skin affection, 2nd group was injected with PRP after the affection; each group contains 20 animals. A separate group of animals served as intact control.

Experimental procedures

Affection of the skin was induced by potassium bichromate (PB) rubbing into nuchae region during 21 day. PRP injections conducted into the dermal layer on the 8th and 15th days after the last PB rubbing (volume – 0,1 ml per injection). PRP was isolated from whole blood with the processing unit SmartPrep (Harvest Corp.). Control mice were injected with the same volume of isotonic solution of NaCl as the animals of the 2nd group. The first day of the experiment was considered the last introduction of PB or PRP in the respective groups. At the end of experiment, all animals were anesthetized by inhalation of the ether vapor. The

anesthetized animals were sacrificed on the 10th, 20th and 30th day and part of skin were removed for the histopathological investigation.

Histopathologic evaluation

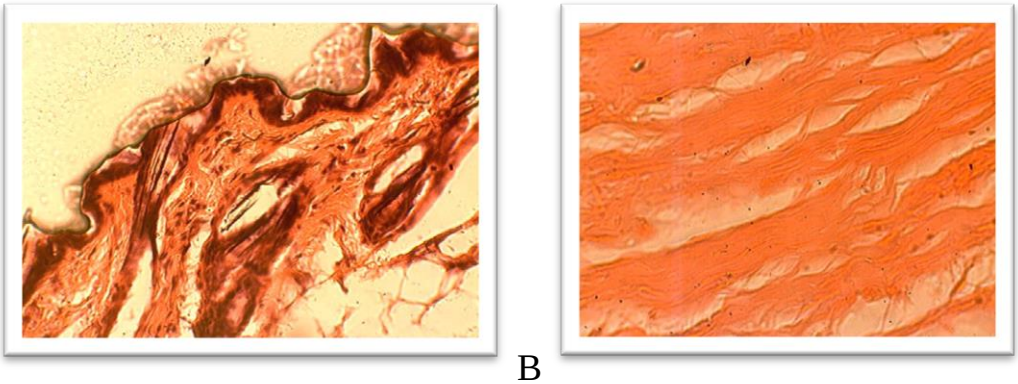
The skin specimens were embedded in the paraffin blocks after they had been fixed in 10 % neutral formalin solution. Sections of 5 μm were obtained, deparaffinized and stained with hematoxylin and eosin (H&E), and Van Gison. The skin was examined and evaluated in random order with standard light microscopy with the light-optical microscope LEICA-DMLS.

Results

In the epidermis of control group all layers are well defined. Both in basal and granular layers dividing cells are present. Underlying derma contains blood vessels, moderate amount of fibroblasts and fibers.

1st day of the experiment

The surface of the skin in both experimental groups is covered by erosions. In the skin signs of acute inflammation with prominent exudation, edema, microvesicles are present (Fig.1 A, B). In the epidermis around vesicles is prominent inter- and intracellular edema, diskeratosis, exocytose as well as acantosis. The vesicles are separated from each other by thin septa, thus forming multi-chamber structures (Fig.2). Within the vesicles lymphocytes, neutrofils, eosinophils and epidermal cells are present.



A B

Figure 1. The skin of experimental animals after rubbing potassium dichromate: A – acute inflammation, B – swelling. Staining: Van-Gison technique, × 200.

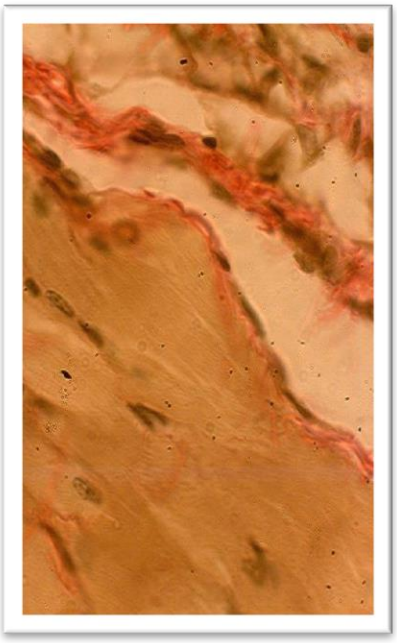


Figure 2. The skin of experimental animals after rubbing potassium dichromate: intraepidermal vesicles formation. Staining: Van-Gison technique, × 400.

In the dermis plethora, affection of vascular wall with areas of perivascular hemorrhages, as well as perivascular infiltrates represented by lymphocytes, histiocytes, with a dash of segmented leukocytes are found. Endothelial hyperplasia is prominent, edematous violation of structural organization of fibrils, dystrophic changes of fibroblasts, fiber fragmentation, sclerosis of vascular walls, swelling are also observed.

10th day of the experiment

In the skin of the 1st group subacute inflammation with the average severity of hyperemia appeared as well as the large number of erosions. Crusts and soak of moderate severity, bleeding ulcers and thinning of the skin were observed (Fig.3). Microscopic changes are characterized by spongiosis, intracellular edema, vesicles of smaller size than at the start of the experiment. Moderate acantosis, hyperkeratosis and prominent intraepidermal infiltration by both lymphocytes and leucocytes were observed (Fig.4).

Figure 3. The skin of the 1st group on the 10th day of the experiment: thinning and desquamation of the superficial epithelium. Staining: H&E, × 100.

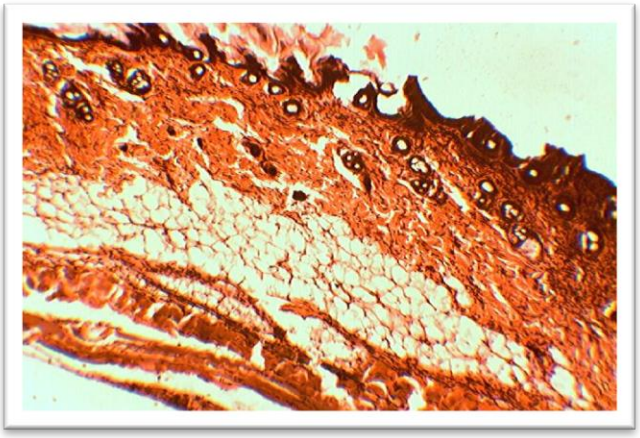


Figure 4. The skin of the 1st group on the 10th day of the experiment: signs of hyperkeratosis. Staining: Van-Gison technique, × 200.

In the 2nd group of animals subacute dermatitis, without hyperemia, small amount of crusts and erosion appear. There is a tendency to cover the skin surface by vellus hairs. Epidermis and dermis slightly thinned due to reduced infiltration. In the presence of single papilla determined vesicles of moderate spongiosis and fibrosis, small foci of parakeratosis. In the papilla the presence of single vesicles and small foci of parakeratosis, as well as moderate fibrosis are determined. At the previous places of vesicles moderate development of fibrous connective tissue is observed.

20th day of the experiment

In the 1st group a slight hyperemia of the skin is present. The contact surface is covered with erosions, solitary soaking. Marked areas of excoriation, lichenification and minimal manifestations are visible. Surface is dry, covered with scales and particles desquamated crusts. In the epidermis perivascular edema with clear boundaries is observed (Fig.5); in the infiltrate lymphocytes, eosinophils and histiocytes are present. In some cases manifestation of degenerative changes of the stratified squamous epithelium is characterized by the epidermal outgrowths and exocytose. In the dermis, changes are characterized by perivascular edema, whose content is represented by lymphocytes and eosinophils.

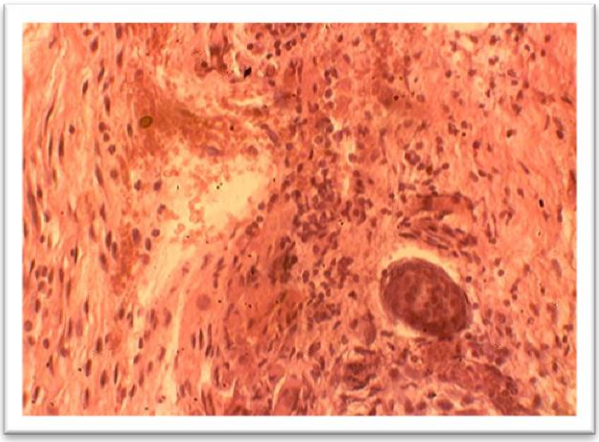


Figure 5. The skin of the 1st group on the 20th day of the experiment: tissue infiltration. Staining: H&E, × 400.

In the 2nd group there were single erosions covered by crusts. Skin surface is dry, without excoriations. The presence of abundant hair growth is determined at the contact place. Under the microscope no spongiosis, acanthosis or dyskeratosis revealed, connective tissue bundles are determined (Fig. 6). In all samples, the change of the dermis is characterized by rich vascularisation.

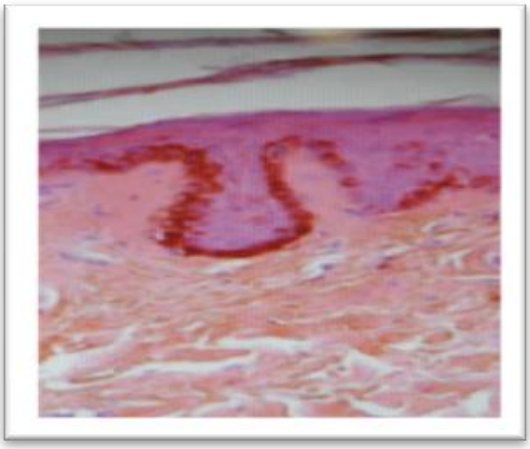


Figure 6. The skin of the 2nd group on the 20th day of the experiment: well defined structures of the skin. Staining: H&E, × 200.

30th day of the experiment

In the 1st group the skin surface is dry, crusts and excoriation remained. There is uneven hair growth on the affected surface due to the presence of scarring of the skin. Epidermis is thinned (Fig.7). The basal membrane of the epidermis is thinned, defragmented. Granular layer is focally presented, spongiosis reaches it's upper layers. In the dermis swelling is reduced, excessive development of fibrous connective tissue at the site of vesicles is present. There is the infiltration by microhistiocytes with areas of epithelioid cells and single giant cells. In dermis swollen dysfibrosis, dystrophic changes of fibroblasts, fibril fragmentation, sclerosis of the walls of blood vessels are revealed (Fig.8).

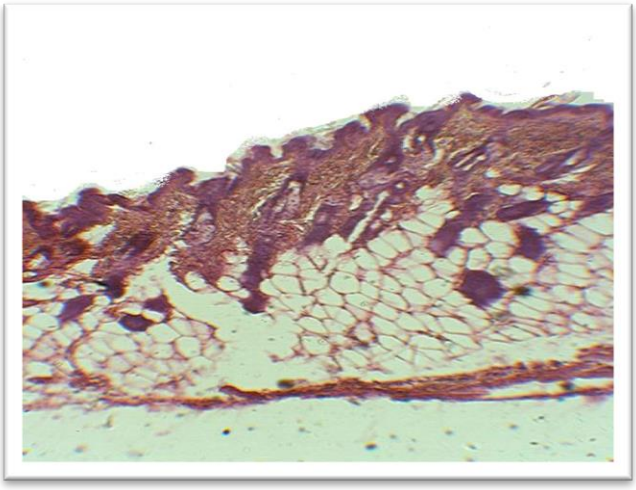


Figure 7. The skin of the 1st group on the 30th day of the experiment: thinning of the epithelium, focal appearance of granular layer. Staining: H&E, × 100.

Figure 8. The skin of the 1st group on the 30th day of the experiment: fragmentation of the fibrils, swollen dysfibrosis. Staining: Van-Gison technique, × 400.

In the 2nd group the skin after affection doesn't differ from the neighboring tissue. Thus, contact area is completely covered with hair. All layers are determined in the skin (Fig.9). Basal and papillary layers are well differentiated what indicates an adequate regeneration process. Dermis is rich in blood vessels and fibroblasts. Skin appendages are of correct morphology.

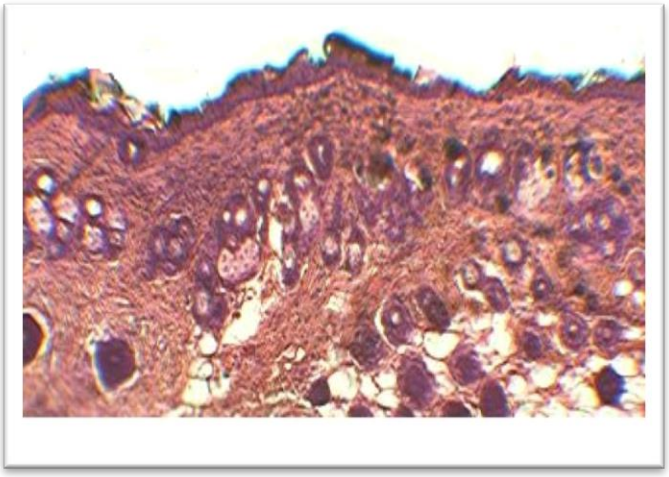


Figure 9. The skin of the 2nd group on the 30th day of the experiment: all layers are present, well differentiated basal layer. Staining: H&E, × 100.

Discussion

PRP promotes qualitative and quick reparative regeneration of the skin, supporting the morphological properties of tissue and organ. Intensity of the regeneration process shows the skin is an organ where tissue processes are combined with each other. This is managed by a number of factors such as cytokines, interleukins and other biologically active substances released from platelets, enhancing chemotaxis and proliferation of cellular elements in the lesion and play an active role in the processes of adaptation [4]. In addition, the fibrin produced in consequence of introduction of PRP forms a kind of natural scaffold, which nests stem cells recruited from their depot by released cytokines.

Acknowledgements

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