PharmacologyOnLine

Archives • 2021 • vol.2 • 84-88

EFFECT OF EXOGENOUS INTRODUCTION OF BLOOD GROWTH FACTORS TO ENHANCE THE HEALING PROCESSES OF SLOWLY HEALING BURN INJURIES

Kucherenko M.P.[1] *; Strus O.E.[4]; Nasibullin B.A.[2]; Gushcha S.G.[2]; Kovalchuk L.I.[1]; Glushchenko V.Y.[1]; Bahrii L.A.[1]; Bobro E.V.[1]

[1]State Institution "South Ukrainian National Pedagogical University named after K. D. Ushynsky", Odesa, Ukraine;
[2]State Institution «Ukrainian Research Institute of Medical Rehabilitation and Resort Therapy of Ministry of Health of Ukraine», Odesa, Ukraine;
[3]State Enterprise "Ukrainian Research Institute of Transport Medicine of the Ministry of Health of Ukraine", Odesa, Ukraine.
[4]Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

*docnickolask@gmail.com.

Abstract

In an experiment on the 40 male white Wistar rats, the authors evaluated the possibility of the influence of the exogenous introduction of blood growth factors on the rate of healing of slowly healing burn injuries. The exogenous supply of blood growth factors to the healing zone was carried out by double administration of platelet-rich plasma.

The research results showed that the additional supply of blood growth factors to the wound area does not affect the intensity of the inflammatory process in it. But, at the same time, the number of fibrous fibers and fibroblasts in the wound increases, the structure of the vessels is restored, the number of neutrophils decreases; the intensity of reparative processes increases significantly. Such changes in the intensity of reparative processes provide a decrease of the wound area, an improvement of its appearance, and less involvement of the surrounding tissues.

Keywords: *burn injury, reparative processes, platelet-rich plasma (PRP).*

Introduction

To date, thermal injuries make up a significant part of the structure of injuries [1]. Non-fatal burns are one of the leading causes of prolonged hospitalization, disfigurement, and disability [2, 3, 4]. Severe complications of burns are long-term non-healing wounds and forming rough scars.

From the point of view of the life quality of the victims, these complications are very significant, because complicate or impair the function of relevant part of the body [5, 6].

The wound healing processes in general and thermal wounds, in particular, are accompanied by many typical reactions: inflammation, cell migration, proliferation, regeneration of the connective tissue, synthesis of non-specific proteins, remodeling of different connective tissues formations [7]. The healing process is cyclical and staged.

The platelet growth factors are very active in the thermal injury healing zone. Long-term non-healing wounds with the subsequent formation of rough scars are most often localized in areas with poor blood supply and trophism, complicated by the presence of an infection resistant to antibiotic therapy, reinfection [8].

Purulent-necrotic dendrite filling the area of long-term non-healing thermal injury contains, among other things, bioactive compounds released during platelet destruction, which affect the course of proliferation and migration of connective tissue cells [9]. In cases of the prolonged healing wound defect, it can be assumed that the amount of released platelet growth factors is insufficient for the optimal rate of formation of a connective tissue (not coarse) scar and epithelialization of the wound. It can be assumed that the introduction of an additional amount of platelets into the body can have a positive effect on the speed and quality of the healing of a burn injury. Platelet-rich plasma can be considered as a carrier of an increased platelet count.

Purpose of the work was to evaluate the effect of injected PRP on the rate of the healing of long-term thermal skin damage.

Methods

The material of this work was the data obtained in the study of clinically healthy male rats (Wistar line), age 7-8 months. The requirements of Directive 2010 \ 63 \ EU European Parliament and of the Council of Europe dated 22.09.2010 "On the protection of animals used for scientific research" and the order of the Ministry of Education and Science, Youth and Sports of Ukraine № 249 of 01.03.2012 was taken into account at the work with the animals.

Under the objectives of this work, the animals were ranked into 2 groups:

1 group - 20 animals, which have been formed a long-term non-healing thermal wound (control)

2 group - 20 animals, which were injected with PRP against the background of the long-term non-healing thermal wound.

A burn injury was created on the lateral surface of the rat's body, previously freed from wool, by applying a red-hot metal disk with a diameter of 13 mm for 3-4 seconds. The injuries were inflicted after rat anesthesia with chloroform.

Platelet-rich plasma was obtained by centrifuging 5.0 ml of fresh blood, obtained from the decapitated rats under light essential anesthesia. The blood was centrifuged at a speed of 2000 rpm for 2 minutes, after which the plasma was taken. The resulting plasma was centrifuged at 4000 rpm in the course of 15 minutes. After that, the top 1/3 of the plasma was taken. The remaining plasma was injected in an amount of 0.1 ml in the burn zone. In rats, after the introduction of PRP, iso-hemagglutination did not occur, i.e. animal blood was compatible. The concentration of platelets in the preparation was 2500 -3000x10^9/l.

The introduction of plasma was performed twice: a day after the burn and on the 4th day after the burn. The duration of the experience is 10 days. To form a non-healing wound, the formed scab was removed daily under ether anesthesia with a non-sterile scalpel.

A day before the end of the experiment, an imprint was obtained from the surface of the wound on a glass slide. It was fixed in alcohol-ether vapors for 6 hours and stained with hematoxylin-eosin. The resulting histological specimen drug was investigated under the light microscope. In 10 fields of view, the number of neutrophils, histiocytes, lymphocytes, fibroblasts were counted.

The animals were removed from the experiment by decapitation under ether anesthesia. Part of the wound was excised closer to the edge. The resulting material was passed through alcohols of increasing concentration and poured into celloidin. Histological preparations with a thickness of 7-9 microns, stained with hematoxylin-eosin, were made.

Preparations were investigated under a light microscope. The data obtained by counting fingerprints were statistically processed using the confidence factor and tabulated.

Results

When studying the state of animals in the group with an uncorrected burn injury, by the end of the experiment (10th day), the surface of the wound was covered with dull granulations, fibrin deposits, the surrounding tissues were edematous, hyperemic and dense on palpation.

The area of the wound corresponds to the diameter of the traumatic element. In the central part, there is a grey, apparently purulent plaque with inclusions of whitish fibrin formations.

Microscopic examination of the bottom of the wound determines a large amount of homogeneous detritus of a pale eosinophilic color. Separate and bundled fibers are identified in the detritus massif. They have different thicknesses and lengths, some of them have fuzzy (blurry) edges. Also, neutrophils are found in large numbers, lymphocytes in much smaller numbers, and histiocytes. The Vessels at the bottom of the wound is either surrounded by neutrophils or have an enlarged perivascular space. The vessels themselves are partially full-blooded, partially spasmodic, the endothelial lining is not readable on all vessels.

The results of the study of smears - prints from the surface of the wound of rats with an uncorrelated course of the wound process are shown in Table 1.

According to table 1, the main part of the cellular composition of the wound is made up of neutrophils. Obviously, this is due to the abundance of necrotic detritus.

The second-largest group of cells is lymphocytes and fibroblasts.

In general, we can say that the cellular composition of the wound contents corresponds to a sluggish inflammatory process.

Discussion

The study of the wound process in animals of the main group, which were injected with PRP against the background of a burn, revealed differences from the state of the wound in the control group of animals.

First of all, an improvement in the general condition of the animals was noted: they actively moved around the cage, had a good appetite, and a rather well-groomed appearance.

Examination of the wound revealed that its area had decreased to 6.0-7.0 mm^2. The surrounding tissues are pale pink, soft, swollen on palpation. The surface of the wound is covered with grayish-pinkish granulations with small deposits of fibrin. A grayish-yellowish bloom remains only in the center of the wound, the edges are free from it.

Histological examination of the bottom of the wound showed that the content of the basic substance is much less than in an uncorrected burn wound, but the number of cellular elements and fibrous fibers increased.

At the same time, the bulk of cells visually contains in close proportions neutrophils, lymphocytes and histiocytes. Among the fibrous fibers, there are quite numerous fibroblasts with oval, well-stained nuclei. But most fibrous fibers are

long and dense. Vessels at the bottom of the wound are predominantly of moderate and increased blood filling, the endothelial lining is complete in the overwhelming majority of the vessels.

Cytological examination of smears-prints of the wound contents (table 1) in rats treated with PRP revealed differences from the wound contents of control animals.

According to table 1, the number of neutrophils decreased by half, the number of fibroblasts, as well as the number of epitheliocytes increased by 3 times. The increase in the number of the latter is evidently due to desquamation and diapedesis of damaged cells during vascular repair. The number of lymphocytes remains close to the values of the control group. It can be assumed that changes in the cell composition of smears-prints corresponds to an inactive inflammatory process and an intensive process of regeneration of the wound bottom tissues.

The results obtained indicate that the introduction of PRP into the zone of a long-term healing burn wound provides an additional supply of blood growth factors to this zone. An additional amount of these factors did not affect the intensity of the inflammatory process in the zone of the burn wound, but increased the volume and speed of reparative processes in it, i.e. there was an increase in the activity of the regeneration process.

Acknowledgments.

The authors declare that there are no conflicts of interest.

References

1. James SL, Lucchesi LR, Bisignano C, et al. Epidemiology of injuries from fire, heat and hot substances: global, regional and national morbidity and mortality estimates from the Global Burden of Disease 2017 study. Inj Prev 2020;26:i36–i45. doi:10.1136/injuryprev-2019-043299.
2. Jeschke MG, van Baar ME, Choudhry MA, et al. Burn injury. Nat Rev Dis Primers. 2020;6(1):e11. doi:10.1038/s41572-020-0145-53/.
3. Yunusova YR., Shapovalov SG, Sukhoparova EP, Besedina EI. Epidemiology of burn trauma in metropolis. The Scientific Notes of Pavlov University. 018; XXV (3): 35–42. doi: 10.24884/1607-4181-2018-25-3-35-42.
4. Kaddoura I, Abu-Sittah G, Ibrahim A, Karamanoukian R, Papazian N. Burn injury: review of pathophysiology and therapeutic modalities in major burns. Ann Burns Fire Disasters. 2017;30(2):95-102.
5. Spronk I, Legemate C, Oen I, van Loey N, Polinder S, van Baar M. Health related quality of life in adults after burn injuries: A systematic review. *PLoS One*. 2018; 13(5):e0197507. doi: 10.1371/journal. pone.0197507.
6. Zhang X, Liu Y, Deng X, Deng C, Pan Y, Hu A. The Correlation Between Quality of Life and Acceptability of Disability in Patients With Facial Burn Scars.Frontiers in Bioengineering and Biotechnology. 2019;7:329. doi:10.3389/fbioe.2019. 00329.
7. Shpichka, A., Butnaru, D., Bezrukov, E.A. et al. Skin tissue regeneration for burn injury. Stem Cell Res Ther. 2019;10:e94. https://doi.org/10.1186/s13287-019-1203-3.
8. Chulak OL, Gozhenko AI, Chulak YuL, Chulak LD, Badiuk NS. Morphology of thermal burn Shpichka, A., Butnaru, D., Bezrukov, E.A. et al. Skin tissue regeneration for burn injury. Stem Cell Res Ther. 2019; 10:e94. https://doi.org/10.1186/s13287-019-12033.
9. Chulak OL, Gozhenko AI, Chulak YuL, Chulak LD, Badiuk NS. Morphology of thermal burn injury under the use of amarant oil (amaránthus). PhOL – PharmacologyOnLine. 2021; 1:1–5.
10. Eisinger F, Patzelt J, Langer HF. The Platelet Response to Tissue Injury. Front Med (Lausanne).2018;5:e317.doi:10.3389/fmed.2018.00317.

Table 1. Cellular composition of the content of the wound when using platelet-rich plasma

Group	Indicators				
	Neutrophilia	Histiocytes	Lymphocytes	Fibroblasts	Epitheliocytes
Group 1	62,5%	20,5%	5,3%	10,2%	1,5%
Group 2	30,2%	29,3%	5,8%	30,2%	4,4%