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MORPHOLOGY OF THERMAL BURN INJURY UNDER THE USE OF AMARANT OIL (*AMARÁNTHUS*)

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Abstract

The authors in an experiment on 54 white rats of the Wistar line, outbred dilution weight 180-200 grams found positive effects of amaranth oil application on morphological processes in thermal burns. The authors found that under the action of amaranth oil there is an early cleansing of the wound from the remnants of necrotic tissue and no purulent exudate is formed. The use of amaranth oil provides the formation of prevention of mass coarsening of fibrous fibers and their detection in the wound was short-lived. In addition, in the wound and the surrounding border area the number of small vessels increased. The process of epithelium crawling on the defect area was intense. Normalization of the activity of redox enzymes was observed. The authors attribute the positive effect of amaranth oil to high content of squalene in it.

Key words: *burn injury, amaranth oil, structural and functional characteristics of the skin*

Introduction

Burn injury occurs when the skin and underlying tissues are damaged due to thermal, chemical, electrical or radiological exposure. Depending on the nature of the damaging factor, thermal, chemical, electrical, and radiation burns are distinguished [1, 2]. Thermal burns determine tissue damage that occurs under the influence of carriers of high temperatures and are observed in fires, inflammation of highly flammable substances, boiling water, hot steam. Coagulation necrosis, serous, serous-fibrinous or purulent inflammation are observed in the tissues damaged by thermal burns. In thermal burns, there are not only local disorders, but also general systemic changes that unite under the name of burn disease [3, 4, 5]. Systemic changes in burn disease cause many numerous and long-term disturbances of homeostasis, which lead to dysfunction of organs and systems. Burn disease and related dysfunctions are determined in 20-40% of cases, depending on the depth of the burn [6].

The development of thermal damage in the early stages is associated with a violation of the first stages associated with impaired hemodynamics, not only local where there is a massive plasma outlet and the corresponding vasospasm can cause changes in plasma and blood and hypoxia in the area of injury.

Violation of hemodynamics in the area of burn injury, along with metabolic intoxication and violation of oxygen-substrate supply creates conditions for violation of the optimal course of healing of burn injury, including the emergence of gross fibrous changes and increase the duration of the process.

Modern principles of treatment of burn wounds treatment include administration of systemic stimulants of fibrosis; elastic compression physiotherapy treatment; local therapy of ulcerative defects. It should be noted that the number of prescribed drugs and conservatives is quite significant and counts hundreds of names, which indicates lack of effectiveness and need to find new remedies.

One of the bioactive factors of natural origin that attracts the attention of researchers is Amaranth (*Amaránthus*) or more precisely, its oil.

The peculiarity of this substance (the presence of amino acids, trace elements, bactericidal compounds) and the presence of a significant amount of squalene, suggests that this remedy can have a positive effect on the course of burns and prevent complications of reparative process. However, in the available literature, we did not find data on the effect of amaranth oil on the healing process in burn injuries. Based on the above, **the aim** of the work was to determine the features of structural and functional changes in the tissues of burn injury under the influence of external application of Amaranth oil.

Methods

54 white rats of the Wistar line of outbred breeding were the material of the study. 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 were taken into account at the work with the animals.

The animals were ranked into 3 groups.

Group 1 included 12 animals that were kept in a vivarium and were not exposed to any influences. The data obtained from them were used as controls.

Group 2 consisted of 21 rats which under ether anesthesia inflicted a burn injury on the lateral surface of the torso.

Group 3 contained 21 rats, which on the background of burn injury applied amaranth oil to the damaged area every day, starting from the first day after thermal damage. The course lasted 10 days.

Thermal injury was inflicted by applying for 10 seconds a coin heated to red color to the shaved side of the body of the animal. The animal was under ether anesthesia herewith. The experiment lasted 10 days. The healing process was evaluated on the 3rd, 7th and 10th days after burns. Amaranth oil was applied externally on the surface of the wound and its edge by 4-5 drops.

On the 3rd, 7th and 10th dyas from the beginning of the experiment, the animals were removed from the experiment by decapitation under ether anesthesia. When removing the rats from the experiment, two pieces of skin that contained the

tissues of the affected area and the intercostal area between the injury and undamaged skin were taken. One piece was fixed with a 4% solution of paraformadehyde, passed through alcohols of increasing concentration, poured into celloidin. Histological sections 7-9 μm thick were made, which were stained with hematoxylin eosin. The second piece was frozen with dry carbon dioxide (t = - 44 °C), cryostat sections 11 μm thick were made, on which the activity of SDH and LDH was determined according to Lloyd's instructions. Evaluation of enzymes activity was carried out semi-quantitatively. Examination of histological specimens was performed using a light microscope.

Results

Macroscopic examination of the burn wound revealed that from the 1st to the 7th days of the experiment, the wound was covered with a hard mountain scab of necrotized tissues, in ≈ 30% of cases, purulent fluid (2-3 drops) is squeezed out from under the scab. Around the scab there is a roller protruding above the surface. At first it is red, then pale pink. At the end of the experiment, the scab is soft, easily separated from the wound, and granulation with a grayish plaque is observed under it.

The use of amaranth oil significantly changed the course of wound nutrition. Already on the 3rd day, the scab covering the wound was translucent, cinnamon in color, i. e. a full rejection of necrotized tissues was present. In no case visible purulent discharges were squeezed out from under the scab. At the end of the experiment, the scab is easily removed, its sizes visually were much smaller than in necrotized healing. After removing the scab almost smooth grayish-pink surface was under it.

Microscopic studies also determined the difference in the course of processes in the wound with uncorrected healing and the use of amaranth oil.

At uncorrected healing at the bottom of the wound for the 3rd day two layers could be visually separated. Directly under the scab a homogeneous eosinophilic mass with a moderate number of lymphocytes was determined. Deeper in this mass bundles of myocytes, pale with pyknosis of nuclei, were determined. Bundles and coarse, short fibrous fibers, lymphocytes and small vessels were full of blood, perivascular edema was present. The number of vessels is quite moderate. Subsequently, in the deep layer, the number of fibrous fibers increased, they remained disordered, some of them were of medium and considerable length (visually). There was also a moderate number of vessels with fibroblasts around them. A certain number of hair sheaths was determined. At the end of the experiment (the 10th day) the above picture was complemented by a significant increase in fibrous fibers forming tracks. Besides we noted the absence of lymphocytes, the presence of round cell elements with rounded nuclei, located rather poorly on the surface.

The microscopic morphology in rats receiving amaranth oil and in rats those with uncorrected wound process were different

At the beginning of the process, it is possible to separate three layers in the bottom. Superficial (under the scab) was a layer of homogeneous eosinophilic substance with a small number of lymphocytes, later it thinned and at the end of the experiment it was not separated. Deeper was a layer consisting of bundle of fibrous fibers arranged in a disordered manner and not a large number of single fibrous fibers. Scattered myositis, pale fibrous fibers were also indentified there. Around small vessels here were fibroblasts in moderation. In the future the number of fibrous bundles, vessels, myocytes collected in bundles increased in this layer, the intermediate substance was small amount. At the end of the experiment a small number of fibroblasts were determined in this layer. Fibrous bundles were long, the interstitial substance was located in fields, vessels had moderate blood supply, and they were quite significant.

At the end of the experiment it was impossible to separate this layer, as it merged with the third layer. The latter layer at the beginning of the experiment contained bundles of fibrous fibers, myositis, mostly normal in appearance and moderate blood supply. Later on, this layer became wider and partially absorbed the second layer. At the end of the experiment, the fields with a similar mesh organization of fibrous bundles were identified in it, they were either pale pink or yellow. Vessels formed loops of lymphocytes and single sebaceous glands were met.

In the case of uncorrected healing it was possible to separate epidermis and skin in the border roller.

In the last 3 days of the experiment, a large amount of interstitial tissue, short coarse scattered fibrous fibers, fibroblasts partly with ordinary oval nuclei, partly with pyknotic nuclei were determined. Few vessels were observed, they had high blood supply. The hair sheaths located here had an enlarged outer layer, which consisted of cells with enlarged juicy nuclei. Epidermis on a roller had a basal layer, the latter consisted of sparse basal cells with juicy colored nuclei. Other layers were not clear.

Later on the surface of the skin itself, wide and flat nubs were formed with a vessel loop in the middle around which fibroblasts and short coarse hair were gathered. In the hair follicles observed, the outer layer is widespread and cells with juicy nuclei, which partially extend over the skin itself are present. In the epidermis, the layer of basal cell cells consists of disordered cells with juicy nuclei, some of them pass to the surface of the wound bottom.

At the end of the experiment in the skin of the navel a large number of bundles of fibrous fibers, which are generally disordered, but where nowhere form reticular structures, basal cell cells quite actively pass out on the wound surface. The intermediate substance is of a moderate amount in the form of individual fields. Vessels are small in number, some of them form loops, their blood supply is moderate. In the epidermis, the layer structure is defined, the basal layer is thickened, part of the basal cells penetrate under the scab. There are cells with enlarged juicy nuclei, other layers are thin, normal in appearance.

In cases when amaranth oil was used for thermal burn healing epidermis and the skin itself were clearly separated in the roller. In the latter there were preserved fibrous bundles, their length was average, the fibers were not coarsened largely. The color of the fibers was moderate, there was an accumulation of fibroblasts around the vessels, the intermediate substance was moderately eosinophilic. Located in the skin, pilares had a thickened outer layer, the cells of which were characterized by enlarged juicy colored nuclei. Lymphocytes were grouped around the hair follicles in moderation. In the epidermis, in contrast to uncorrected wound healing, the basal layer was formed by basal cells with rounded succulent nuclei located in a disordered multi-row variant.

The spiked layer consisted of sparse cells with flattened nuclei, the granular layer was not readable, keratin one was represented by separate plates. Subsequently, in the skin itself, fibrous bundles form reticular-like structures (fields), at the end of the experiment, these fields merged. Visually, fibrous fibers were almost indistinguishable from the pattern of control skin. The outer layer of the hair follicles was thickened due to the increase in cell nuclei. The skin itself formed flat pips but the vessels of the loop were not in all pips. The vessels of the skin itself were moderately full-blooded. The intermediate substance was contained in moderation, so the skin itself, in contrast to the uncorrected process, was not wide. At the end of the experiment, the adjacent to the damage and covering the scab thins and part of it (thinned) entered under the scab. The epidermis on the roller was usually layered. The basal layer between the navels was multicellular, in other areas it was single-row. All other layers were readable, but they were not wide. In the part of the skin that was under the scab, the epidermis was represented

The results of histoenzymogenic studies.

On the 3rd day of the experiment, the activity of SDH in basal cells of the border roller was 5.0 ± 0.14 U, in fibroblasts of the bottom of the wound it substituted 3.0 ± 0.154 U; in the cells of the outer layer of the pilaris - 5.0 ± 0.124 U. LDH activity in basal cells was 4.0 ± 0.34 U; in cells of an external layer of hair - 4,0 ± 0,12 and in fibroblasts of a bottom of a wound - 4,0 ± 0,344 U. That is, there was some weakening of the activity of redox enzymes, but the ratio of their activity corresponded to the control data.

Later on, the activity of the enzymes under study increased. To the 7^{th} day of the experiment LDH activity in the basal cell of the roller was 6.0 ± 0.14 U; in the cells of pilaris it was 6.0 ± 0.074 U; in bottom fibroblasts - 4,0 ± 0,14 U. LDH activity in basal cells was 6.0 ± 0.144 U; in the cells of pilaris is equaled to 6.0 ± 0.14 U; in fibroblasts -4,0 ± 0,124 U.

At the end of the experiment, a further increase in the activity of the redox enzymes was observed. SDH in the roller basal cells remained at the level of the previous period of study; in the cells of the outer

layer of the pilaris it constituted 7.0 ± 0.54 U; in fibroblasts - 6.6 ± 0.314 U. That is, the redox process was constantly growing.

The use of amaranth oil as a means of correcting wound healing was accompanied by differences in the behavior of redox enzymes. On the 3rd day of the experiment, the activity of LDH in the basal cell of the roller was as in the control and equaled to 6.0 ± 0.17.od; in the cells of the outer layer of the pilaris it was 6.0 ± 0.214 U; in fibroblasts of the wound bottom - 4.0 ± 0.204 U. LDH activity in basal cells was 6.0 ± 0.21 U; bottom fibroblasts - 6.0 ± 0.14 U; in the cells of the pilaris it was - 5.0 ± 0.3 U.

On the 7th day of the experiment, the activity of the redox enzymes increased. SDH activity in basal cells was 7.0 ± 0.194 U; in the cells of the pilaris it equaled to 7.0 ± 0.204 U; in fibroblasts of the wound bottom it increased up to 6.0 ± 0.314 U. LDH activity also increase. In basal cell cells it was 7.0 ± 0.234 U; in fibroblasts - 6.0 ± 0.11 U. At the end of the experiment, the activity of SDH and LDH in the cells under study was equal to the values of control group animals. SDH in basal cell cells was 6.0 ± 0.14 U; in pilaris - 6.0 ± 0.244 U; in fibroblasts - 5.0 ± 0.274 U. LDH activity in basal cells decreased to 6.0 ± 0.44 U; in fibroblasts up to 5.0 ± 0.214 U; in the cells of the pilaris decreased to 6.0 ± 0.244 U.

Conclusions

1. Thus, the results of the studies have shown that in the area of thermal damage healing is accompanied by coarsening of fibrous fibers, increase in fibroblasts, a moderate number of vessels, but stagnant plethora; high content of intermediate substance. That is, there are conditions for the formation of complications in the form of a rough scar.

If the wound healing took place under the conditions of amaranth oil treatment, the course of the wound process became special.

First, there was an earlier cleansing of the wound from the remnants of necrotic tissue and the absence of purulent exudate.

Second, the coarsening of fibrous fibers and their lifespan were little -definied and short-lived.

Third, visually, the number of vessels in the border strip and the bottom of the wound when applying amaranth oil was higher.

Fourth, the crawling of the epithelium on the wound surface was intense and quick than at the uncorrected process.

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The authors declare that there are no conflicts of interest.

References

1. Nader Aghakhani, Hamid Sharif Nia, Mohammad Ali Soleimani, Nasim Bahrami, Narges Rahbar, Yadegar Fattahi, Zahra Beheshti. Prevalence burn injuries and risk factors in persons older the 15 years in Urmia burn center in Iran. Caspian J Intern Med. 2011 Spring; 2(2): 240–244
2. Ruth, A.; Bryant, D.P.N. Acute and Chronic Wounds, 4th ed.; Elsevier Inc.: St. Louis, MO, USA, 2010..
3. Sen, C.K.; Roy, S. Redox signals in wound healing. Biochim. Biophys. Acta 2008, 1780, 1348–1361.
4. Toshihiro Kurahashi, Junichi Fujii. Roles of Antioxidative Enzymes in Wound Healing.J. Dev. Biol. 2015, 3, 57-70; doi:10.3390/jdb3020057
5. Roy, S.; Khanna, S.; Nallu, K.; Hunt, T.K.; Sen, C.K. Dermal wound healing is subject to redox control. Mol. Ther. 2006, 13, 211–220. J. Dev. Biol. 2015, 3 68
6. Ibrahim A.E., Sarhane K.A., Fagan S. P., Goverman J. renal dysfunction in burns: a review. Ann Burns Fu\ire Disaster, 2013, Mar 31; 26(1): 16-25