Theoretical and Experimental Cryobiology

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ASSESSMENT OF HYALURONIC ACID AND DMSO INFLUENCE ON LOW-TEMPERATURE TISSUE DAMAGE COURSE: AN EXPERIMENTAL MODEL

The research performed a local histological assessment of tissue tolerance of a solution containing hyaluronic acid, as well as its effect on the course of the inflammatory process after low-temperature skin damage was investigated. The safety of using a 1% solution of high molecular weight hyaluronic acid with the addition of 5% dimethyl sulfoxide (DMSO) and its effect on the inflammation development was studied using a model of low-temperature tissue injury based on the results of clinical observation, planimetric and histological studies. Additionally, the effect of 1% solutions of high molecular weight (> 2,000 kDa) and low molecular weight (10—100 kDa) hyaluronic acid on the inflammatory process after low-temperature tissue injury was comparatively explored. According to the results of the histological study, no morphological signs of toxic or other negative effects of subcutaneous administration of a 1% solution of high molecular weight hyaluronic acid with the addition of 5% DMSO on tissues were found. Comparison of the effects of cryoprotective solutions on the course of the wound process after low-temperature tissue damage revealed the differences both by the severity of macroscopic signs of the inflammation and by planimetric study data. The inclusion of hyaluronic acid in the composition of the cryoprotective solution is considered a optimistic approach to reducing the concentration of DMSO in order to create solutions that can be used without a washing-out stage.

Key words: *hyaluronic acid, cryoprotective solution, low-temperature tissue damage, skin, wounds, cryoprotectant, cryopreservation.*

Hyaluronic acid (HA) is a natural polysaccharide with pronounced hydrophilic properties, widely used in biotechnology and practical medicine due to its anti-inflammatory, regenerative and antioxidant properties [2, 8, 10, 16]. It was first isolated from the bull eye vitreous [26], and later found to be present in the extracellular matrix of most nervous and connective tissues [9]. Due to the presence of a carboxyl group in each HA fragment, it has a natural negative charge, which provides the ability to absorb large amounts of water and swell up to 1,000 times its volume [24, 25].

The number of studies devoted to the use of HA in cryobiology is limited and has ambiguous conclusions [17, 27, 29, 33]. Such discrepancies may be associated with the significant dependence of the HA properties on its molecular weight, that always necessitates the need for additional comparative

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experiments. The molecular weight of HA remains a key factor determining the diversity of physicochemical characteristics and biological activity of this substance [34].

High molecular weight HA (HMW HA) is known to have anti-inflammatory properties, while low molecular weight HA (LMW HA) promotes im-mune stimulation and enhances inflammatory processes [7, 20, 30]. In previous research [11] the authors have already reported the regenerative properties of HMW HA and LMW HA solutions in an excision wound model and showed that they were more pronounced for LMW HA than for HMW HA solutions. The ability of HA molecules to bind a significant amount of free water opens up broad prospects for its use in technologies related to the application of low temperatures [3, 18, 23, 28]. Reducing the amount of free water in cryopreserved biological objects contributes to the inhibition of crystal formation and growth of ice crystals during freezing and thawing, which, in turn, decreases mechanical damage to cells [19, 21]. Such properties make HA a promising component of a cryoprotective solution. The cryoprotective solution largely determines the final result of preserving the viability of a cryopreserved biological object. Recently, special attention has been paid to the trend of reducing the percentage of toxic cryoprotectants in favor of their partial replacement with less harmful substances that additionally have certain cryoprotective properties. Leveling the toxic effect of the components is primarily necessary for cryoprotective solutions that do not require washing-out. Hyaluronic acid, as a substance of natural origin, is one of the promising components for its study as an additional component of such solutions. There is currently a significant share of research investigating the effect of gelling agents in cryo-protectant solutions on cryopreservation efficiency. Particular attention is paid to natural gelling agents, such as HA, which is capable of vitrification at moderately low temperatures. HA is most often used in combination with dimethyl sulfoxide (DMSO) during freezing [28]. DMSO is a synthetic organic compound that penetrates well through skin and cell membranes and is able to transport other substances into deep tissue layers. This property can be useful in medicine, but at the same time it increases the risk of toxic effects, especially at high concentrations. DMSO can cause protein denaturation, disrupt the integrity of cell membranes and even promote apoptosis. In addition, it is partially converted in the body to dimethyl sulfide, which can have additional toxic effects and cause an unpleasant odor.

Current research was aimed at finding additives to DMSO-based cryoprotectant solutions, the addition of those reduced its initial concentration and created a cryoprotectant solution composition that will be much safer, less toxic and will not re-quire washing after the thawing procedure [5, 31]. The need to wash biological objects after exposure to standard DMSO solutions remains a significant limitation: this increases the procedure duration, requires special conditions, and is also accompanied by the loss of part of the biological material and a decrease in its functional quality. The proposed solution with the addition of HA potentially allows minimizing these shortcomings, combining the effectiveness of cryoprotection with the safety of further use of the biomaterial.

At the stages of cell cultivation and incubation before freezing, HA is used as an additional component of the corresponding solutions [23, 29] with subsequent freezing in DMSO solution. Recently, more and more studies have been published confirming the positive effect of adding HA directly to the composition of the cryoprotective solution based on traditional cryoprotective agents [3, 12, 22, 36].

There are numerous reports that highlight the separate use of DMSO or HA for wound healing of various etiologies [1, 15]. Much less research is devoted to the treatment of cold injuries directly, especially with the use of DMSO. There are singles reports on the treatment of frostbite of animal skin [14]. However, it should be noted that both substances are used in combination with other substances: HA as a gelling agent, and DMSO as a carrier for drug delivery [32].

Despite the natural origin and long experience of using HA in medicine and cosmetology, its use requires additional study, in particular regarding the effect on tissues after low-temperature injury. Low-temperature tissue damage is characterized by a complicated pathophysiology, since the lesion affects not only the surface layers of the skin, but also deeper structures — vessels, muscles and nerves. This type of traumas is accompanied by the rapid development of pronounced edema, that makes them a convenient experimental model for studying the anti-inflammatory effect of solutions containing HA.

The purpose of the work was to determine the local tissue tolerance of a hyaluronic acid solution and 5% dimethyl sulfoxide according to histological research and to assess its effect on the course of the inflammation after low-temperature skin injury.

MATERIALS AND METHODS

The experiments used 5% DMSO (USA) and 1% HA of different molecular weight (Bang&Bonsomer, Finland): low molecular weight (LMW HA; 10—100 kDa) and high molecular weight (HMW HA; >2,000 kDa) in saline.

The research design was approved by the Bioethics Committee of the Institute of Cryobiology and Cryomedicine of the NAS of Ukraine (Protocol No. 5 dated 11/22/2022). The experiments were conducted in accordance with the Law of Ukraine "On the Protection of Animals Against Cruelty" and the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986).

The experiment was performed in hairless rats kept under standard conditions of the Animals' House of the IPCC of the NAS of Ukraine.

For anesthesia, the drug "Telazol" (Zoetis, USA) was used (50 mg/kg of rats' weight, intramuscularly).

Animals were removed from the experiment by decapitation after anesthesia, with subsequent histological control of the state of the tissue in the zone of cold injury on the 14th and 28th days of the study.

The material was fixed in 10% aqueous neutral formalin solution containing buffer, then celloidin-paraffin embedding was performed, after which serial sections with a thickness of 4—5 × 10^{-6} m were prepared with a microtome-cryostat "MK-100" (EKA, Ukraine). Hematoxylin and eosin-stained slides were used for general assessment of tissue condition. Picrofuscin staining according to the Van Gieson and Mallory methods was used to detect and assess the development of connective tissue structures. Iron hematoxylin staining was applied to determine alternative changes in the muscle tissue of the thigh of rats. Histological and histochemical methods were performed in accordance with the histological technique and histochemistry manuals [35].

Histological preparations were studied using an Olympus BX-41 microscope (Olympus, United Kingdom) and Olympus DP-Soft 3.1 software (Olympus). Evaluation was performed in at least 10 randomly selected fields of view.

Low-temperature tissue damage was simulated by pressing a cryoapplicator (8.0 mm diameter, −196 °C temperature) to the thigh lateral surface of both paws for 30 seconds. Immediately after cryoapplication, around the visible area of skin injur and partially under the damaged skin, the test solution was injected in a volume of 0.8 ml from 6—8 points, retreating 1—2 mm from the injured area visible border. Thus, the HA formed a "cushion" around and under the damaged skin. Injections were performed only in the rats' right paw, while the left paws remained control.

The dynamics of wound healing was observed for 28 days, performing weekly photofixation of the wound surface current state. The wound area on days 1, 7, 14 and 28 was determined by the planimetric method using the open-source software "ImageJ 1.54" (National Institutes of Health, USA).

The study was conducted in several stages. First, the feasibility of including HA in cryoprotective solutions that do not require washing-out was determined, in particular, the safety of local administration of HMW HA with DMSO. After confirming safety, its anti-inflammatory effect, expected based on the biological properties of HA, was tested. The model of low-temperature tissue damage was chosen due to the notable edema that occurs after exposure to low temperatures. To exclude the effect of DMSO, the ones of separate administration of HMW HA were studied. To assess the effect of the molecular weight of HA on wound healing after low-temperature skin damage, that of separate administration of LMW HA was studied.

To identify the possible toxic effect of the cryoprotective solution (1% solution of HMW HA with the addition of 5% DMSO) upon subcutaneous administration, the histological structure of thigh tissues of intact rats and those with injections of the solution was evaluated at the first stage.

Design of the research first stage.

Group 1.1 (control) — intact animals, $n = 7$. Group 1.2 — subcutaneous injection of 1% solution of HMW HA with the addition of 5% DMSO, $n = 7$.

At the second stage of the work, the effect of a 1% solution of HMW HA with 5% DMSO on the development of the inflammatory process in animals after low-temperature tissue damage was studied based on clinical observation. To justify the choice of the composition of the cryoprotective solution, the effect of solutions containing HMW HA or LMW HA on the inflammatory process after

low-temperature tissue damage was separately com-pared.

Design of the research second stage.

Group 2.1 (cryoapplication) — low-temperature damage with no solutions introduced, $n = 21$.

Group 2.2 (cryoapplication + HMW HA + + DMSO) — low-temperature damage and subcutaneous injection of a 1% solution of HMW HA with 5% DMSO, $n = 7$.

Group 2.3 (cryoapplication + HMW HA) — low-temperature damage and subcutaneous injection of a 1% solution HMW HA, $n = 7$.

Group 2.4 (cryoapplication + LMW HA) — low-temperature injury and subcutaneous injection of 1% LMW HA solution, $n = 7$.

A total of 35 animals were used in the study.

Statistical data processing was performed using the program "Statistica 10.0" (StatSoft, USA) using the nonparametric Mann–Whitney test and the Kruskal–Wallis test. The data were presented as M + m, where M is the mean value, m is the standard deviation. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Due to the fact that the therapeutic effect of HA largely depends on its molecular weight, the primary task was to determine the optimal composition of the HA solution for further use in cryopreservation. The fact that after cold injuries there is always an inflammation with pronounced edema in the area of injury, and DMSO is often added to the composition of the cryoprotective solution as a cryoprotectant, was taken into account. In addition, it is HMW HA that has a pronounced anti-inflammatory effect [7, 13], and DMSO is known for its anti-inflammatory properties [6].

The research first stage. Study of the effect of the cryoprotective solution of HMW HA and DMSO on the histological structure of the thigh tissues of experimental animals when administered subcutaneously.

Microscopic examination of tissues of intact rats (group 1.1, control) revealed epidermis, dermis and muscle tissue in the thigh tissues (Fig. 1). The epidermis was represented by a multilayered squamous keratinized epithelium with three layers: basal, spinous and horny.

The basal layer of the epidermis consisted of a single row of flattened cells with an oval intensely basophilic nucleus and an average degree of chromatin dispersion; mitotic figures were observed in some places. The spinous layer contained 1—2 rows of smaller polygonal cells with a moderate ability to perceive basic dyes. The *stratum corneum* consisted of 1—2 rows of anucleate eosinophilic horny scales. The basal membrane of the epidermis was continuous, thin, homogeneous.

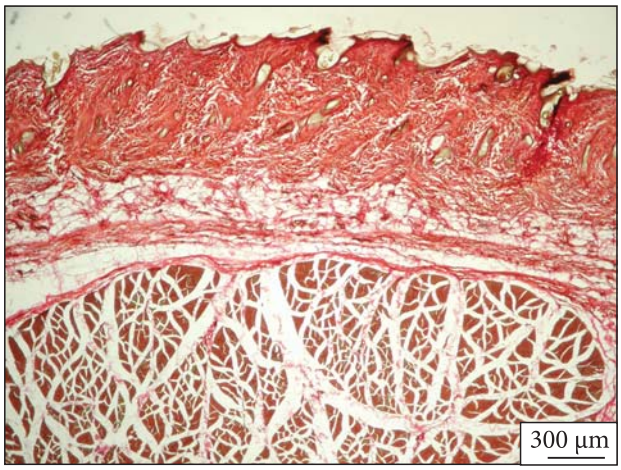

Fig. 1. Normal histological structure of rat's thigh tissues. Group 1.1 (control). Van Gieson staining, ×40

The papillary and reticular layers were visualized in the dermis. The papillae of the dermis were wide, flattened, represented by loose fibrous connective tissue. When staining according to Van Gieson and Mallory, thin collagen and elastic fibers were determined. Capillaries and a few cells (fibroblasts, macrophages, tissue basophils, lymphocytes) were located between the connective tissue fibers.

The reticular dermis contained intensely fuchsinophilic bundles of collagen fibers with a network of elastic fibers oriented parallel to and at an angle to the skin surface. Fibroblasts and fibrocytes with moderately to weakly basophilic nuclei and weakly eosinophilic cytoplasm were occasionally visualized in the main substance.

Skin appendages (hair follicles and sebaceous glands) were few. Epithelial cells of the hair sheath had a large rounded basophilic nucleus and moderately acidophilic cytoplasm. Sebaceous glands consisted of large cells with optically empty or light cytoplasm and a large rounded weakly basophilic nucleus. The epithelium basement membrane of the hair sheaths and sebaceous glands was thin and continuous.

Capillaries of the papillary and reticular layers had a well-defined lumen with a moderate number of formed blood elements. Endotheliocytes had a flattened shape, weakly basophilic cytoplasm and

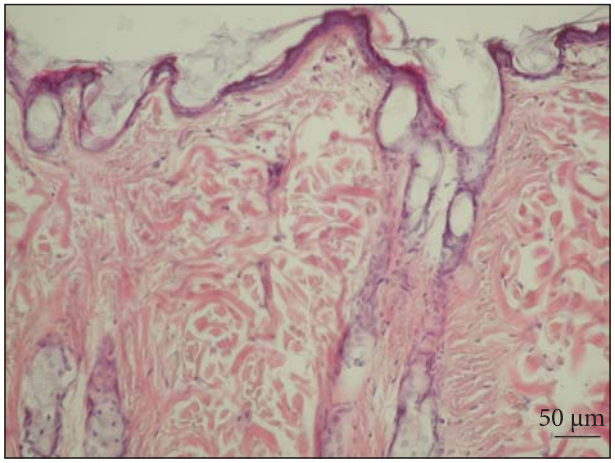

Fig. 2. Normal histological structure of rat's epidermis and dermis. Group 1.1 (control). Hematoxylin and eosin staining, ×200

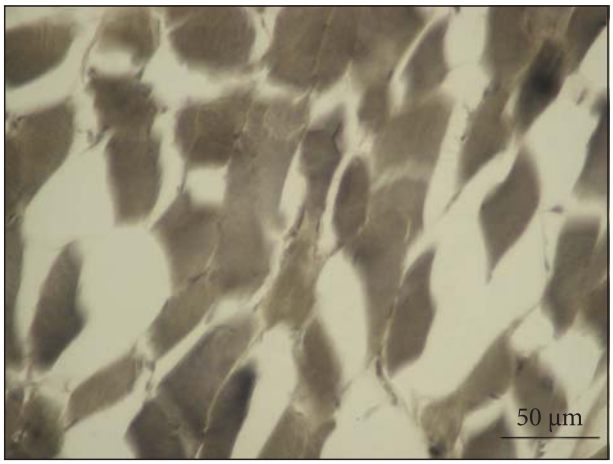

Fig. 3. Muscle fibers of rat's thigh muscles. Group 1.1 (control). Iron hematoxylin staining, ×400

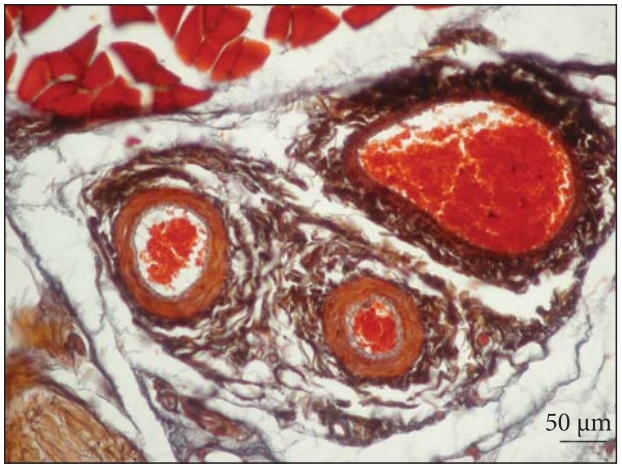

Fig. 4. Neurovascular bundle in the intermuscular space of rat's thigh. Group 1.1 (control). Mallory staining, ×200

a slightly elongated basophilic nucleus, and were located on a thin continuous vascular membrane. Finely focal lymphomacrophage infiltrates were visualized around the capillaries in places (Fig. 2).

A thin layer of adipose tissue islets was found in the hypodermis, with thin bundles of fuchsinophilic collagen fibers with small vessels and nerve trunks passing between them.

Muscle tissue consisted of fibers with moderately or weakly basophilic rod-shaped nuclei and eosinophilic cytoplasm. When stained with iron hematoxylin, muscle fibers had a gray color (Fig. 3). Between the fibers were layers of loose fibrous connective tissue with thin fuchsinophilic collagen fibers, few fibroblasts, vessels, nerves (Fig. 4). Small arteries and veins of the hypodermis and muscle layer were moderately blood-filled; endothelial cells of the arteries formed a palisade, the basement membrane was thin, continuous. Veins were somewhat tortuous, dilated, full-blooded. The nuclei of endothelial cells were clear, moderately basophilic, located close to each other.

Thus, the histological structure of the thigh tissues of intact rats corresponded to the norm.

Microscopic examination of thigh tissues of rats of group 1.2 (subcutaneous administration of HMW HA with DMSO) on the 14 and 28th days revealed no traumatic or inflammatory changes. The epidermis retained a normal structure: basal epidermocytes were flattened with oval basophilic nuclei, the *stratum corneum* consisted of 1—2 rows of anucleate scales, the basement membrane was thin and continuous (Fig. 5).

In the dermis, papillary and reticular layers were determined without pathological changes (Fig. 6). Cell elements were represented by fibroblasts and fibrocytes with weakly eosinophilic cytoplasm and weakly basophilic nucleus. Capillaries had a noticeable lumen with mainly erythrocytes, endothelial cells were flattened, the basement membrane was thin, continuous. Small perivascular lymphomacrophage infiltrates were somewhere determined.

The hypodermis contained layers of adipose tissue separated by thin fuchsinophilic collagen fibers extending from the reticular dermis. Between them small vessels and nerve trunks ran.

Muscle fibers retained transverse striation, rod-shaped nuclei moderately or weakly basophilic. When stained with iron hematoxylin, muscle fibers were gray (Fig. 7). Small arteries of the hypodermis and muscle layer were moderately filled with blood.

Veins had a somewhat tortuous shape, the lumen was dilated, filled mainly with erythrocytes. Endotheliocytes had a clear moderately basophilic nucleus. The basement membrane of arteries and veins was thin, continuous. Thin fuchsinophilic layers of collagen fibers were visualized between nerve fibers.

Thus, in a comprehensive study of microphotos of thigh tissues of animals of group 1.2 (after subcutaneous administration of a solution of HMW HA with DMSO) on the 14th and 28th days, the histological picture, the severity degree of histochemical responses in the epidermis, dermis, hypodermis and thigh muscles did not differ from group 1.1 (control). Thus, according to the results of the histological study, no morphological signs of toxic or other negative effects of subcutaneous administration of a 1% solution of HMW HA with the addition of 5% DMSO on tissues were detected.

The results of the first stage became the basis for testing the assumption regarding the anti-inflammatory effect of HA in the model of low-temperature tissue injury.

The research second stage. The effect of cryoprotective solutions containing HMW HA with 5% DMSO, either HMW HA or LMW HA on the inflammation development after low-temperature tissue damage was studied.

First of all, the effect of a 1% solution of HMW HA with 5% DMSO on the development of the inflammatory process was evaluated according to clinical observation. Additionally, the effect of 1% solutions of HMW HA and LMW HA on the inflammatory process after low-temperature tissue damage was compared.

When the skin is damaged, complex interactions occur between different types of skin cells, peripheral nerve, as well as immune and vascular cells [4].

It is known that the inflammatory phase begins immediately after tissue injury. It is characterized by typical vascular reactions in the area of injury: first, vasoconstriction occurs, followed by vasodilation, the release of formed blood elements into the affected area (hemorrhages are visible in Fig. 8), fibrin loss and infiltration of surrounding tissues by cell elements of inflammation with a clear demarcation of the area of injury. Regeneration begins with the formation of a fibrin matrix and fibronectin. Even within the first 10 hours after injury, collagen synthesis starts, getting the main structural component of the wound matrix and giving the strength to the resulting scar. Maximum collagen

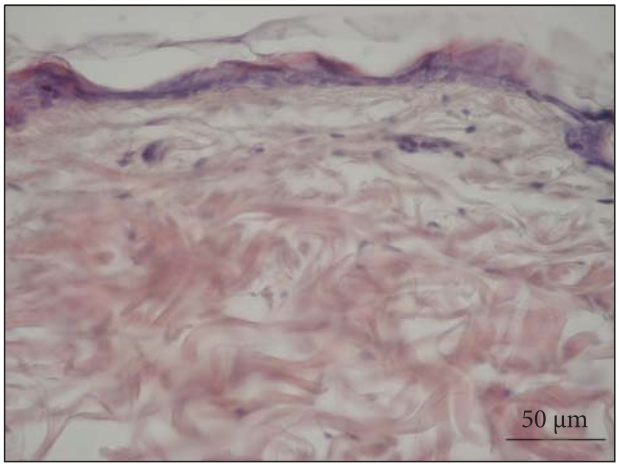

Fig. 5. Continuous layer of normal epidermis in the injection area. Few fibroblasts in the dermis papillary layer. Group 1.2 (subcutaneous injection of 1% solution of HMW HA with 5% DMSO), day 14. Hematoxylin and eosin staining with, ×200

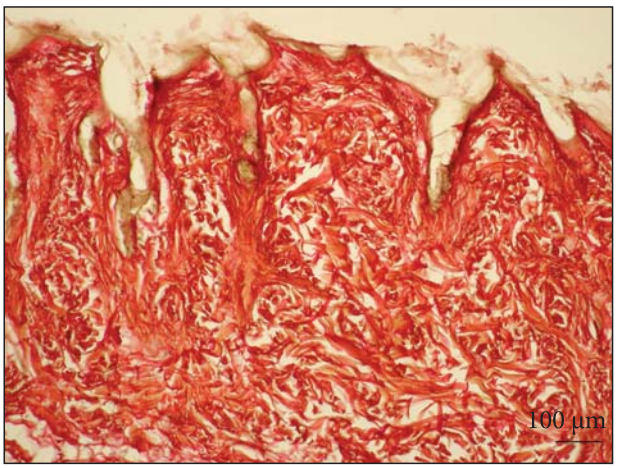

Fig. 6. Fuchsinophilic collagen fibers of the dermis papillary and reticular layers. Group 1.2 (subcutaneous injection of 1% solution of HMW HA with 5% DMSO), day14. Van Gieson staining, ×200

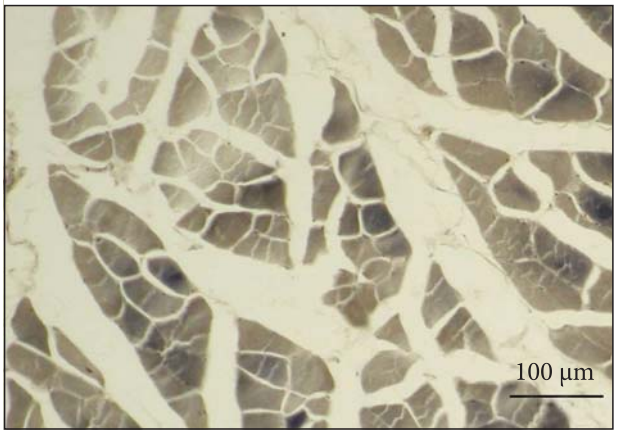

Fig. 7. Muscle fibers of the thigh muscles of rats. Group 1.2 (subcutaneous injection of 1% solution of HMW HA with 5% DMSO), day 28. Iron hematoxylin staining, ×200

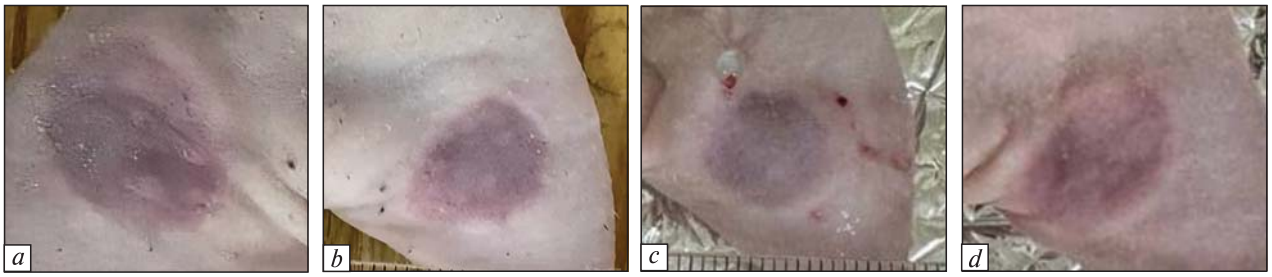

Fig. 8. Macroscopic photo of the low-temperature injury zone 24 hours after cold injury: *a* — with no additional solutions introduced (group 2.1), *b* — introduction of HMW HA with DMSO (group 2.2), *c* — introduction of HMW HA (group 2.3), *d* — introduction of LMW HA (group 2.4)

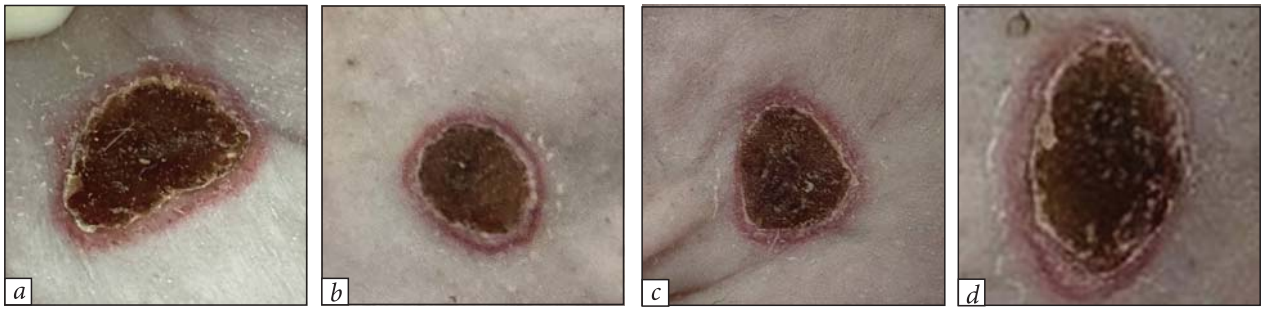

Fig. 9. Macroscopic photo of the low-temperature injury zone on the 7th day of observation: *a* — with no additional solutions introduced, *b* — introduction of HMW HA with DMSO (group 2.2), *c* — introduction of HMW HA (group 2.3), *d* — introduction of LMW HA (group 2.4)

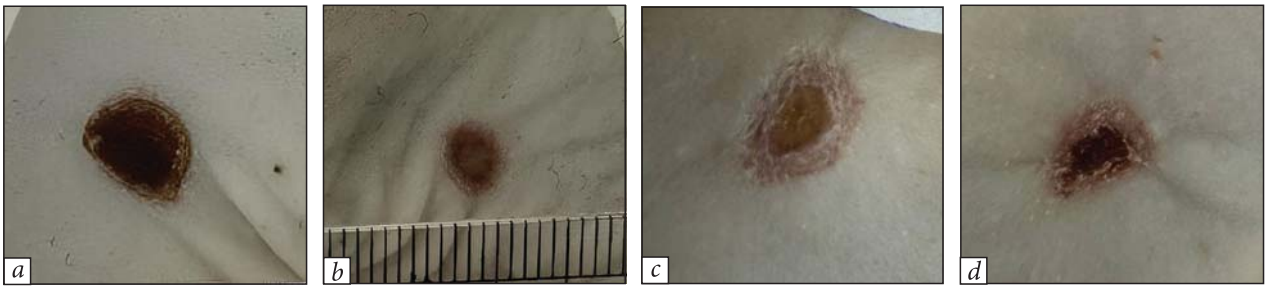

Fig. 10. Macroscopic photo of the low-temperature damage zone on day 14 of observation: *a* — with no additional solutions introduced (group 2.1), *b* — introduction of HMW HA with DMSO (group 2.2), *c* — introduction of HMW HA (group 2.3), *d* — introduction of LMW HA (group 2.4)

production is observed on the 5th—7th days, afterwards its level gradually decreases. By the day 3, fibroblasts appear, and they become the dominant cell type in this phase. Cytokines secreted by macrophages simultaneously stimulate vascular proliferation, which provides fibroblasts with oxygen and nutrients, accelerates cell growth and supports the synthesis of the wound matrix. Fig. 8, *a* shows the appearance of the low-temperature injury zone without the introduction of solutions (group 2.1 — control) 24 hours after the application of cold injury.

At this time, a zone of injury with pronounced edema and tissue ischemia was observed on the skin. The skin in the center of the injury acquired a blue-violet color, lost elasticity and became dense. The peripheral zone looked like a pale corolla without a clear border with the central area. Visual observation data indicate significant microcirculation disorders in the zone of low-temperature exposure to the skin of animals, which emphasizes the important role of vascular factors in tissue damage by low temperatures.

Fig. 8, *b* shows the zone of low-temperature injury with subcutaneous administration of 1% HMW HA with 5% DMSO (group 2.2) 24 hours after injury. Subcutaneous injections of the solution after low-temperature exposure were accompanied by a decrease in macroscopic signs of edema and

tissue ischemia. According to planimetric research, the area of wounds after the solution injection was 1.28 times smaller versus the control group.

On the day 7, the lesion area in the control group had the appearance of a wound with a dark brown scab and raised edges (Fig. 9, *a*). The peripheral part looked like a dark red corolla with uneven color, which is explained by moderate hyperemia, small foci of hemorrhages and granulation tissue, which shines through the newly formed epithelium. Moderate edema and desquamation of the epithelium were observed around the wound. Therefore, according to the results of visual assessment, it can be stated that there are inflammatory and reparative processes in the tissues along the periphery of the wound.

The introduction of the studied solution had a positive effect on wound healing: hyperemia, hemorrhages and edema decreased (Fig. 9, *b*). The area of the wounds was 1.34 times smaller than in the control group (group 2.1).

On the 14th day in the control group, as for the previous observation period, the wound remained covered with a scab with raised edges (Fig. 10, *a*), local signs of the inflammatory process on the periphery were less pronounced (than on the 7th day), but desquamation of the surface epithelium persisted.

Fig. 11 shows planimetry data comparing the state of the wound surface of group 2.2 (HMW HA + + DMSO) with the control (2.1). The wound area on the 1st and 7th days was: for group 2.1 — (91 ± + 3.4) mm^2 and (71 ± 5.1) mm^2, for group 2.2 — (63 ± 5.8) mm^2 and (47 ± 3.2) mm^2, respectively.

Fig. 12 shows the planimetry for groups 2.3 (HMW HA) and 2.4 (LMW HA). The area of wounds on the 1st and 7th days: for group 2.3 — (73 ± 4.3) mm^2 and (45 ± 4.6) mm^2, for group 2.4 — (82 ± 4.9) mm^2 and (55 ± 3.7) mm^2, respectively.

In agreement with visual assessment and planimetry data, solutions of HMW HA and HMW HA with DMSO suppressed the development of inflammation on the 1st and 7th days more effectively than LMW HA. No differences were noted between HMW HA and HMW HA with DMSO.

Since hairless rats were used in the study, wound epithelialization was marginal in nature: epithelial cells migrated from the wound edges into the tissue defect, which is possible only under the conditions of cleaning the epithelialization zone from necrotic masses and filling it with granulation tissue. Acceleration of wound epithelialization after the administration of HMW HA with DMSO indicates the activation of reparative processes in the zone of low-temperature damage.

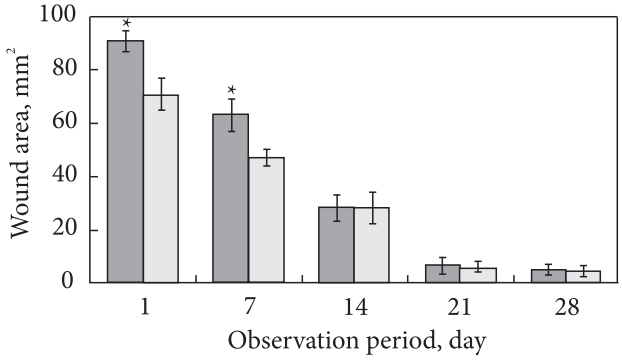

Fig. 11. Dynamics of wound healing after low-temperature tissue injury: ■ — group 2.1 (cryoapplication); □ — group 2.2 (cryoapplication + HMW HA + DMSO); * — significant differences relative to the indices of group 2.1, $p < 0.05$

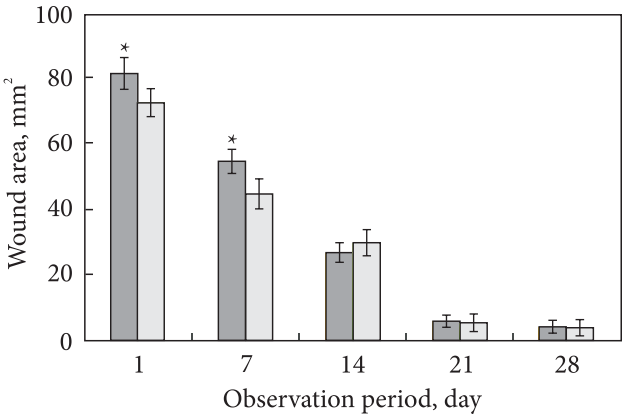

Fig. 12. Dynamics of wound healing after low-temperature tissue injury: ■ — group 2.4 (cryoapplication + + LMW HA); □ — group 2.3 (cryoapplication + HMW HA); * — differences are significant relative to the indices of group 2.3, $p < 0.05$

Injections of HMW HA with DMSO reduced the manifestation of inflammation. Thus, macro-scopic signs of hyperemia or edema of tissues around the wound were not observed, hemorrhages were minimal, desquamation was less pronounced, and the activity of marginal epithelialization was greater than in the control group 2.1. The results of the planimetric study confirmed the presence of significant differences between the indicators in both study groups on the 1st and 7th day (see Fig. 11). The effect of HMW HA with DMSO on the wound area on the 14th, 21st and 28th days was not detected, that requires further study. This is probably due to the pathogenetic features of the wound process after low-temperature injury and can be explained by the effect of HMW HA on such an

important pathogenetic link of inflammation as edema. It is known that it is in the first phase (inflammation) of the wound process that tissue edema in the area of low-temperature injury rapidly develops. The effect of HMW HA, as the authors believe, is implemented through the binding of a significant amount of inflammatory exudate fluid, which contributed to the reduction of inflammation activity in injured tissues. The pathogenetic focus of such an effect is realized by reducing the effect of vascular factors of the damaging effect of low-temperature exposure on biological tissues.

From the point of view of the pharmacological characteristics of the components, the cryoprotective solution of HMW HA with DMSO has anti-inflammatory and antiseptic properties when applied topically, which has been confirmed experimentally. The results obtained prove the prospects of using cryoprotective solutions containing HMW HA and DMSO in cryobiology.

Further studies can be aimed at optimizing the ratio of HA and DMSO to design the cryoprotective solutions that do not require washing-out before use.

CONCLUSIONS

1. Histological study helped to establish that subcutaneous administration of a cryoprotective solution consisting of a 1% solution of HMW HA with 5% DMSO did not cause a negative effect on the structure of tissues in intact animals and did not disrupt the course of the wound process caused by low-temperature skin damage.

2. Clinical observation showed that subcutaneous injections of 1% solution of HMW HA with 5% DMSO into the area of cold-induced injury led to faster tissue recovery, which was manifested by a decreased wound area and an improved general sign of repair.

3. Comparison of the effect of cryoprotective solutions (1% HMW HA, 1% LMW HA or 1% HMW HA with 5% DMSO) on the course of the wound process after low-temperature injury showed significant differences between the groups: on the 1st and 7th days, the area of wounds in the animals injected with solutions of HMW HA and HMW HA with 5% DMSO was smaller compared to the group receiving the solution of LMW HA. No significant differences were found between the groups with HMW HA and HMW HA with 5% DMSO.

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ОЦІНКА ВПЛИВУ ГІАЛУРОНОВОЇ КИСЛОТИ ТА ДМСО НА ПЕРЕБІГ НИЗЬКОТЕМПЕРАТУРНОГО УШКОДЖЕННЯ ТКАНИН: ЕКСПЕРИМЕНТАЛЬНА МОДЕЛЬ

У роботі проведено локальну гістологічну оцінку тканинної переносимості розчину, що містить гіалуронову кислоту, та досліджено його вплив на перебіг запального процесу після низькотемпературного ушкодження шкіри. На моделі низькотемпературного ушкодження тканини вивчено безпеку застосування 1%-вого розчину високомолекулярної гіалуронової кислоти з додаванням 5 % диметилсульфоксиду (ДМСО) та його вплив на розвиток запального процесу за результатами клінічного спостереження, планіметричного та гістологічного досліджень. Додатково в порівняльному аспекті вивчено вплив 1%-вих розчинів високомолекулярної (> 2000 кДа) та низькомолекулярної (10—100 кДа) гіалуронової кислоти на запальний процес після низькотемпературного ушкодження тканин. За результатами гістологічного дослідження не виявлено будь-яких морфологічних ознак токсичного або іншого негативного впливу підшкірного введення 1%-го розчину високомолекулярної гіалуронової кислоти з додаванням 5 % ДМСО на тканини. Порівняння впливу кріозахисних розчинів на перебіг ранового процесу після низькотемпературного ушкодження тканин виявило відмінності як за виразністю макроскопічних ознак запального процесу, так і за даними планіметричного дослідження. Включення гіалуронової кислоти до складу кріозахисного розчину розглядається як перспективний підхід до зниження концентрації ДМСО з метою створення розчинів, які можуть використовуватися без стадії відмивання.

***Ключові слова*:** гіалуронова кислота, кріозахисний розчин, низькотемпературне ушкодження тканин, шкіра, рани, кріопротектор, кріоконсервування.