DOI 10.26724/2079-8334-2025-2-92-184-188
UDC 617-001.4-085-076.5

P.T. Muraviov, M.H. Bespalko[1], B.S. Zaporozhchenko
Odesa National Medical University, Odesa
[1]MUI "Odesa Regional Clinical Medical Center" of the Odesa Regional Council, Odesa

SKIN INJURY SURFACE CYTOLOGY DYNAMICS CHANGES IN EXPERIMENTAL WOUND PROCESS

e-mail: gemostatik@gmail.com

Long-persisting chronic wounds represent a significant challenge for general surgery specialists. The purpose of the study was to determine the characteristics of cellular composition changes along the wound surface during post-traumatic regeneration under the conditions of combined application of collagen hydrolysate in an experimental setting. Experimental studies were performed on the model of Voltran Ekol ES55 pneumatic weapon induced gunshot wound. Collagen hydrolysate was additionally included to complex wounds' therapy. The efficacy of wounds' healing was estimated morphologically and immunohistochemically. The main group rats (13 (43.3 %)) were treated with a collagen hydrolysate, meanwhile the comparison group rats (17 (56.7 %)) – without. On the 7th day, CD45+ infiltrate was expressed in both groups, while CD68+ cells were found in greater amount in the main group. On the 14th day, CD68+ cells were detected in the inlet zone in both groups, but in the main group their amount was lesser. CD163+ cells were verified in both groups as scattered single cells. Verification of CD45+ cells on the 14th day revealed isolated positive cells scattered among fibrous tissue in both experimental groups. On the 28th day quite a few CD68+ cells were detected in the superficial layers of the scar in the main group, that was unsimilar to the comparison one. The authors supposed the data obtained are the experimental evidence of collagen hydrolysate clinical efficacy testing reasonability for wound healing outcomes improving.

Key words: rat, wound, cytology, regeneration, wound process, collagen hydrolysate, wound treatment.

П.Т. Муравйов, М.Г. Беспалько, Б.С. Запорожченко

ДИНАМІКА ЗМІН ЦИТОЛОГІЧНОЇ КАРТИНИ РАНОВОЇ ПОВЕРХНІ В УМОВАХ ЕКСПЕРИМЕНТАЛЬНОГО РАНОВОГО ПРОЦЕСУ

Тривалі хронічні рани є серйозним викликом для спеціалістів загальної хірургії. Метою дослідження було визначення особливостей змін клітинного складу вздовж поверхні рани під час посттравматичної регенерації за умов комбінованого застосування гідролізату колагену в експериментальних умовах. Експериментальні дослідження проводили на моделі вогнепального поранення, спричиненого пневматичною зброєю Voltran Ekol ES55. До комплексної терапії ран додатково включали гідролізат колагену. Ефективність загоєння ран оцінювалася морфологічно та імуногістохімічно. Щурам основної групи (13 (43,3 %)) вводили гідролізат колагену, а щурам групи порівняння (17 (56,7 %)) – ні. На 7-му добу в обох групах виявлено експресію CD45+ клітинного інфільтрату, тоді як в основній групі виявлено більшу кількість CD68+ клітин. На 14 добу CD68+ клітини були виявлені у зоні вхідного отвору в обох групах, але в основній групі їх кількість була меншою. Клітини CD163+ були виявлені в обох групах і мали вигляд поодиноких та розсіяних. Перевірка CD45+ клітин на 14-й день виявила окремі позитивні клітини, розкидані серед фіброзної тканини в обох дослідних групах. На 28 добу в поверхневих шарах рубця в основній групі виявлено досить багато CD68+ клітин, що не відповідало клітинній картині в групі порівняння. Отримані дані автори вважають експериментальним доказом доцільності тестування ефективності гідролізату колагену для покращення результатів загоєння ран в клінічних умовах.

Ключові слова: щур, рана, цитологія, регенерація, рановий процес, гідролізат колагену, лікування рани.

The study is a fragment of the research project "Development of modern methods of diagnosis and treatment of purulentseptic complications in combat surgical trauma", state registration No. 0120U101834.

Long-persisting chronic wounds that do not respond adequately to existing surgical treatment methods represent a significant challenge for general surgery specialists [1, 2]. The complexity and multidirectional nature of the available pharmacological and surgical approaches to wound treatment, along with the controversial issues regarding the timely use of diagnostic studies and the coordinated efforts of specialists from related fields in selecting the most optimal treatment strategy, highlight the relevance and debate surrounding of this issue [1–3, 5, 8].

In recent years, there have been increasing reports of favorable outcomes from pharmacological treatment involving collagen hydrolysate, demonstrating its positive effects on fibroblast proliferation, the restoration of tissue basophil levels, and the organization and maturation of collagen fibers, leading to the formation of dense connective tissue [11, 14]. Special attention is given to surgical interventions at various stages of the wound-healing process to restore the wound surface to a condition that promotes faster healing [6, 10, 12]. However, existing contradictions regarding the potential "manageability" of the wound-healing process in terms of improving and accelerating regeneration necessitate a more thorough investigation of this issue.

The purpose of the study was to determine the characteristics of cellular composition changes along the wound surface during post-traumatic regeneration under the conditions of combined application of collagen hydrolysate in an experimental setting.

Materials and methods. The study of wound healing processes in an experimental setting involved 30 one-year-old Wistar rats, weighing 250–300 grams, without signs of chronic or acute diseases. During the experiment, the animals underwent a 10-day quarantine and were housed under standard vivarium conditions with a complete diet and free access to water.

Animal preparation, all interventions, anesthesia were conducted in full compliance with the requirements of the "Instructions" of the State Pharmacological Center of the Ministry of Health of Ukraine (Kyiv, 2001), as well as the Good Laboratory Practice (GLP) regulations established by the European Commission for overseeing laboratory and other studies, in accordance with the Code of Scientific Ethics of Ukraine.

The injuries were inflicted in a laboratory setting using a Voltran Ekol ES55 pneumatic weapon, simulating a gunshot wound. The animals were immobilized on a wooden board. The wound site was the hind limbs, where the projectile damaged the skin, subcutaneous fat tissue, and muscles, causing minor blood loss without bone damage. The entry wound diameter was 0.3 ± 0.5 mm, with relatively even wound edges in relation to the surrounding tissues. The wound channel length reached 1 cm. No animals died during the whole experimental trial.

The main group included 13 rats (43.3 %) that received additional treatment with collagen hydrolysate. The comparison group consisted of 17 rats (56.7 %) undergoing post-traumatic tissue regeneration without additional intervention. Wound healing lasted for 28 days of the trial was assessed visually and through cytological material collection (imprint smears) from the wound surface. Among the research methods, particular attention was given to immunohistochemical analysis to determine the cellular composition of regenerative tissue zones and to verify vascular structures.

Immunohistochemical reactions were performed using CD45, CD68, CD163, and CD34 markers. For immunohistochemical studies, deparaffinization and antigen retrieval were conducted in a PT module (DAKO, USA) using HIER buffer (pH = 6.0 and pH = 9.0). The immunohistochemical reaction itself was performed in an Autostainer Link 48 ("DAKO," USA) according to the manufacturer's antibody protocol. Visualization was based on the DAKO EnVision+System detection system (DAKO, USA). The following monoclonal antibodies were used: Mo a-Hu CD68 Antigen, Clone 514H12 (Leica Biosystems, Germany); Mo a-Hu CD163 Antigen, Clone BSB 6304 (Bio SB, USA); Mo a-Hu CD34, Clone QBEnd/10 (ThermoScientific, USA); Mo a-Hu CD45, Clone 2B11 (DAKO, USA).

Result evaluation and photodocumentation of all histological stains and immunohistochemical reactions were performed in a standardized field of view using "Leica DM2000" LED microscope with a 20 MP camera (Germany) at magnifications of ×40 (eyepiece ×10, objective ×4), ×100 (eyepiece ×10, objective ×10), and ×400 (eyepiece ×10, objective ×40).

The results were estimated using statistical variation analysis methods with Microsoft Office Excel 2016 software. Statistical analysis of the experimental study results was performed using one-way ANOVA parametric criterion, with differences considered statistically significant at $p<0.01$.

Results of the study and their discussion. Up to the 7th day after injury, both groups of animals exhibited an expressed purulent-necrotic exudate on the wound surface, containing a large number of neutrophilic granulocytes, many of which showed signs of dystrophy, degradation, and lysis. The inflammatory exudate contained minor fragments of hair, scattered granules of amorphous acellular masses (foreign material), and a significant amount of fibrin mixed with rod-shaped and coccal flora.

The peripheral areas of the wound contained a considerable amount of rat hair and amorphous acellular masses in the form of fibers and granules, surrounded by a dense mass of eosinophilic homogeneous protein and degenerating granulocytes. In the wound center, the deeper layers of the exudate showed a tendency toward densification, fibrin homogenization, and "sealing" of inflammatory cells within protein masses—considered early signs of scab or plaque formation over the injury site. Notably, there were no differences in the cellular composition or wound surface characteristics between the comparison group rats.

A study of the deep tissues of the wound channel revealed significant edema in the derma and underlying fat tissue, with collagen fiber separation and the formation of perivascular edema zones. There was also diffuse-focal, unevenly distributed, intense infiltration of inflammatory cells (lymphocytes, neutrophilic granulocytes, macrophages) throughout all elements of the wound channel. The vascular endothelium exhibited signs of "swelling", and in some areas, endothelial changes resembling a "palisade" pattern were observed. Collagen fibers stained intensely eosinophilic with variable intensity.

Regarding the comparison group, no significant differences were observed in the structural changes of the wound channel or in the cellular composition of the inflammatory infiltrate.

A detailed investigation was conducted on inflammatory infiltration zones in relation to collagen fiber formation using Van Gieson's staining. It was found that the areas of inflammatory cell accumulation had relatively clear boundaries, involved lipocytes and blood vessels, and contained almost no collagen fibers.

Upon closer examination, expressed edema with collagen fiber separation was observed. The inflammatory infiltration consisted primarily of neutrophilic granulocytes with a small number of lymphocytes, located around and between lipocytes, which themselves showed clear signs of necrobiosis and necrosis. The cellular composition of the inflammatory infiltrate was dominated by leukocytes. Both membranous and cytoplasmic staining of cells was considered positive, and CD45+ cells included leukocytes such as granulocytes, lymphocytes, eosinophils, basophils, monocytes, macrophages/histiocytes, mast cells, and plasma cells. Notably, CD45+ infiltration was prominent in both groups, not only as part of the inflammation in the wound channel walls but also within the scab covering the entry wound.

Regarding cells of the histiocytic-macrophage lineage, identified using the CD68 monoclonal antibody, a predominance was noted in the main group. As these cells are inherently present in the inflammatory process, they respond to inflammatory stimulation by enhancing the monocyte-macrophage response, which was verified using the CD163 marker. Thus, both groups exhibited a significant presence of macrophages/monocytes within the exudate on the wound surface. When analyzing the cellular composition of inflammation in the wound channel tissues, macrophages/monocytes were documented near the epidermis and around blood vessels, both at the entry site and within the wound channel walls. Their distribution was predominantly diffuse, with a tendency toward perivascular clustering.

To summarize the results of the immunohistochemical study in the experimental group, a quantitative assessment was performed by counting the average number of antibody-positive cells in 20 fields of view at 400× magnification (Table 1).

Table 1

Summary of leukocyte and macrophage-histiocytic cell count in rat wounds (average count in 20 fields of view)

Detected cells subtypes	7th day		14th day		28th day	
	Main group (n=13)	Comparison group (n=17)	Main group (n=13)	Comparison group (n=17)	Main group (n=13)	Comparison group (n=17)
CD45+	28.6±2.1	27.2±2.4	17.4±1.7	16.2±1.8	4.1±0.2*	6.0±0.3*
CD163+	18.8±2.3	19.1±2.1	5.8±0.6	9.1±0.7	3.2±0.2	5.1±0.3
CD68+	14.6±1.8*	15.1±2.1*	4.1±0.3*	8.3±1.1*	2.1±0.2*	5.3±0.4*

Note. * – result of reliability check, p=0.044, t=2.01

A detailed study of the cellular composition of the inflammatory infiltrate in the wounds of rats from both groups was performed. On the 14th day of the experiment, the number of CD45+ cells was slightly lower in both groups.

However, a considerable number of cells with a positive reaction to the common leukocyte antigen were still present both at the wound entry site and within the wound channel walls. These cells were primarily grouped within the newly formed granulation tissue, both among the newly formed microcirculatory vessels and within the connective tissue.

Regarding the proportion of histiocytic-macrophage lineage cells, a positive reaction to CD68 was detected in the wound entry zone of rats from both groups. However, in animals receiving collagen, fewer of these cells were observed (Fig. 1), whereas in the comparison group, their accumulation was documented (Fig. 2).

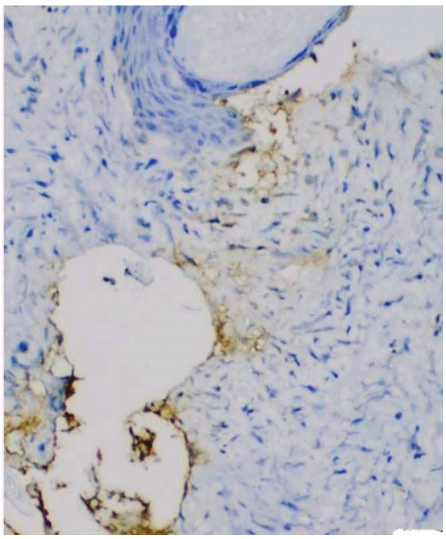

Fig. 1. Localization of CD68+ cells at the wound edges of rats in the main group on the 14th day of the experiment. IHC reaction with CD68, magnification ×200.

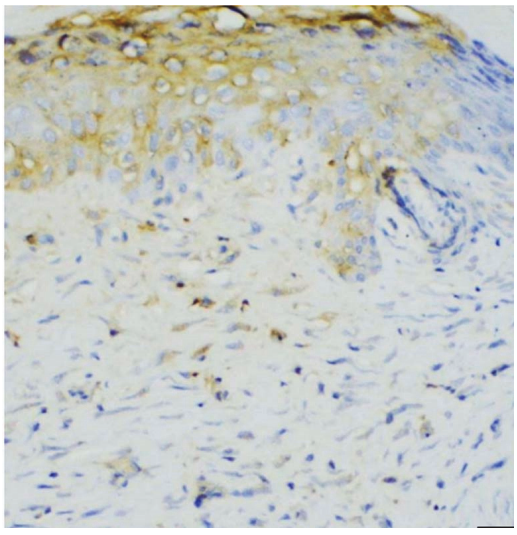

Fig. 2. Localization of CD68+ cells at the wound edges of rats in the comparison group on the 14th day of the experiment. IHC reaction with CD68, magnification ×200.

If we consider the presence of macrophage/monocyte cells at the wound edges, CD163+ cells were verified in both groups as scattered single cells. A quantitative assessment of leukocyte, macrophage-histiocytic, and macrophage-monocytic cells on the 14th day of the experiment is presented above.

Verification of CD45+ cells (i.e., cells expressing the common leukocyte antigen) revealed isolated positive cells dispersed among fibrous tissue in both experimental groups. No grouping of these cells, follicle formation, or tropism towards blood vessels was observed.

A specific reaction with the monoclonal CD68 antibody verified isolated positive cells in the superficial layers of the scar tissue in animals from the main group. However, in the comparison group, there was a significant accumulation of these histiocytic-macrophage cells among the inflammatory infiltrate and fibroblasts, particularly at the border with the regenerating epidermis.

A similar morphological pattern was observed in the IHC reaction with CD163. Macrophages/monocytes appeared as small, sparse clusters around blood vessels, at the epidermal boundary, and perivascularly. However, in this respect, there were no significant differences between the studied groups. The quantitative analysis of inflammatory cell subtypes (leukocytes, macrophage-histiocytic lineage, monocytes/macrophages) was also summarized and presented above.

In discussing the obtained data, we would like to emphasize the positive impact of collagen medicines in wound healing and tissue regeneration. This is evidenced by a statistically significant reduction in the number of CD68+ histiocytic-macrophage cells in the main experimental group starting from day 7, and a decrease in CD45+ cells by day 28th of the experiment. This finding aligns with data from other researchers [9, 11, 13].

The experimental results allow us to underscore the feasibility and potential of collagen-based treatments in clinical practice for patients with chronic, non-healing wounds. Moreover, we consider it necessary to explore the use of collagen preparations in the comprehensive treatment of patients with comorbid conditions, particularly those with complicated type 2 diabetes mellitus, as reflected in recent scientific publications [4, 7, 15]. Based on the obtained results, we are confident that the integration of collagen preparations into wound treatment strategies – aimed at improving and accelerating connective tissue regeneration – will be highly beneficial. This is particularly relevant given the current realities of mine-explosive and gunshot injuries, where such treatments could significantly aid both the general population and individual patients.

Conclusion

The experimental data on the use of collagen hydrolysate highlight its positive effect on wound regeneration processes, as confirmed by the dynamic results of cytological and immunohistochemical analyses of wound surface samples. The observed effects of collagen hydrolysate in the experiment, compared to the control group, open promising opportunities for improving wound healing outcomes in clinical practice.

Prospects for further research aimed at thorough investigation of collagen hydrolysate time-dependent efficacy in experimental wounds' conditions with obvious its recommendation if effective to use in clinical practice.

References

1. Atkin L, Bućko Z, Montero EC, Cutting K, Moffatt C, Probst A. et al. Implementing TIMERS: the race against hard-to-heal wounds. J Wound Care. 2019; 23 (Sup3a): 1-50. doi: 10.12968/jowc.2019.28.Sup3a.S1.
2. Beeckman D, Cooper M, Greenstein E, Idensohn P, Klein RJ, Kolbig N. et al. The role community-based healthcare providers play in managing hard-to-heal wounds. Int Wound J. 2024; 21(1): e14402. doi: 10.1111/iwj.14402.
3. Boersema G, Smart H, Giaquinto-Cilliers MGC, Mulder M, Weir GR, Bruwer FA. et al. Management of non-healable and maintenance wounds: a systematic integrative review and referral pathway. Adv Skin Wound Care. 2021; 34(1): 11-22. doi: 10.1097/01.ASW.0000722740.93179.9f.
4. Burgess J, Wyant W, Abdo B, Kirsner R, Jozic I. Diabetic Wound-Healing Science. Medicina. 2021; 57 (10): 1072. doi: 10.3390/medicina57101072.
5. Dabas M, Schwartz D, Beeckman D, Gefen A. Application of Artificial Intelligence methodologies to chronic wound care and management: A scoping review. Adv Wound Care (New Rochelle). 2023; 12(4): 205-240. doi: 10.1089/wound.2021.0144.
6. Eriksson E, Liu PY, Schultz GS, Martins-Green MM, Tanaka R, Weir D. et al. Chronic wounds: Treatment consensus. Wound Repair Regen. 2022; 30(2): 156-171. doi: 10.1111/wrr.12994.
7. Hu P, Lei Q, Duan S, Fu Y, Pan H, Chang C. et al. In-situ formable dextran/chitosan-based hydrogels functionalized with collagen and EGF for diabetic wounds healing. Biomater Adv. 2022; 136: 212773. doi: 10.1016/j.bioadv.2022.212773.
8. Khomenko IP, Lurin IA, Nehoduiko VV, Tertyshnyi SV, Popova OM, Vastyanov RS. et al. Soft tissue perfusion evaluation algorithm in the scheme of gunshot defects "closure". World of Medicine and Biology. 2023; 1 (83): 169-173. doi: 10.26724/2079-8334-2023-1-83-169-173.
9. Li M, Hou Q, Zhong L, Zhao Y, Fu X. Macrophage Related Chronic Inflammation in Non-Healing Wounds. Front Immunol. 2021; 12: 681710. doi: 10.3389/fimmu.2021.681710.

10. Liu YF, Ni PW, Huang Y, Xie T. Therapeutic strategies for chronic wound infection. Chin J Traumatol. 2022; 25(1): 11-16. doi: 10.1016/j.cjtee.2021.07.004.
11. Mathew-Steiner S, Roy S, Sen C. Collagen in Wound Healing. Bioengineering. 2021; 8(5): 63. https://doi.org/10.3390/bioengineering8050063.
12. Mayer DO, Tettelbach WH, Ciprandi G, Downie F, Hampton J, Hodgson H. et al. Best practice for wound debridement. J Wound Care. 2024; 33 (Sup6b): 1-32. doi: 10.12968/jowc.2024.33.Sup6b.S1.
13. Pang J, Maienschein-Cline M, Koh T. Monocyte/Macrophage Heterogeneity during Skin Wound Healing in Mice. J Immunol. 2022; 209 (10): 1999–2011 doi: 10.4049/jimmunol.2200365.
14. Sharma S, Rai VK, Narang RK, Markandeywar TS. Collagen-based formulations for wound healing: A literature review. Life Sci. 2022; 297: 120436. doi: 10.1016/j.lfs.2022.120436.
15. Wang Y, Chen Li, Ren DY, Feng ZX, Zhang LY, Zhong YF. et al. Mussel-inspired collagen-hyaluronic acid composite scaffold with excellent antioxidant properties and sustained release of a growth factor for enhancing diabetic wound healing. Mater Today Bio. 2022; 15: 100320. doi: 10.1016/j.mtbio.2022.100320.

Стаття надійшла 17.06.2024 р.

DOI 10.26724/2079-8334-2025-2-92-188-192
UDC 615.322: 615.276

O.O. Nefodov, L.V. Eberle[1], O.V. Ustyanska, A.O. Tsisak, O.I. Alexandrova, I.M. Radaeva, K.V. Ostapchuk[1]
Odessa I.I. Mechnikov National University, Odessa, [1]Odessa National Medical University, Odessa

EVALUATION OF THE ANTI-EXUDATIVE EFFECT OF OINTMENT WITH ONONIS SPINOSA L. EXTRACT IN MODELS OF INFLAMMATION OF VARIOUS ORIGIN

e-mail: lidaeberle@gmail.com

Experimental studies on the antiexudative activity of Ononis spinosa L. extract were conducted in models of trypsin and zymosan-induced inflammation. To assess the activity of the plant extract, ointments of varying concentrations (1.5 %, 2.5 %, 3.5 %) in terms of polyphenolic compounds were prepared for transdermal application. According to the study's results, the 3.5 % ointment exhibited the most pronounced anti-exudative effect, effectively suppressing the focus of inflammation and contributing to an accelerated reduction in the morphological indicators of the affected limbs in rats. The ointment with Ononis spinosa L. extract did not differ significantly, but was not inferior in effect to the comparison drug - Dolgit cream. The obtained data indicate the feasibility of using Ononis spinosa L. extract in the treatment of inflammatory processes, particularly those of infectious origin. The identified pharmacological activity justifies the need for further preclinical and clinical studies. The results obtained may serve as the basis for the development of effective local anti-inflammatory agents of plant origin.

Key words: antiexudative effect, inflammation, Ononis spinosa L. extract.

О.О. Нефьодов, Л.В. Еберле, О.В. Устянська, А.О. Цісак, О.І. Александрова, І.М. Радаєва, К.В. Остапчук

ОЦІНКА АНТИЕКСУДАТИВНОЇ ДІЇ МАЗІ З ЕКСТРАКТОМ ONONIS SPINOSA L. НА МОДЕЛЯХ ЗАПАЛЕННЯ РІЗНОГО ҐЕНЕЗУ

Проведено експериментальні дослідження антиексудативної активності екстракту Ononis spinosa L. на моделях трипсинового та зимозанового запалення. З метою оцінки активності рослинного екстракту були виготовленні мазі різних концентрацій (1,5 %, 2,5 %, 3,5 %) в перерахунку на поліфенольні сполуки для трансдермального нанесення. Згідно результатів дослідження встановлено, що найбільш виражену антиексудативну дію проявляла 3,5 % мазь, яка ефективно пригнічувала осередок запалення та сприяла пришвидшеному зменшенню морфологічних показників уражених кінцівок щурів. Мазь з екстрактом Ononis spinosa L. суттєво не відрізнялась, та не поступалась дії препарату порівняння – Долгіт крему. Отримані дані свідчать про доцільність використання екстракту Ononis spinosa L. у терапії запальних процесів, зокрема інфекційно-опосередкованого ґенезу. Виявлена фармакологічна активність обґрунтовує потребу в подальших доклінічних і клінічних дослідженнях. Отримані результати можуть стати основою для створення ефективних місцевих протизапальних засобів рослинного походження.

Ключові слова: антиексудативна дія, запалення, екстракт Ononis spinosa L.

The study is a fragment of the research project "Pharmacological correction of simulated pathological conditions through the use of developed drugs", state registration No. 0122U200545.

Inflammatory processes are among the most common pathological conditions that accompany both acute and chronic diseases, affecting various organs and body systems. They arise in response to tissue damage, exposure to toxic substances, infectious agents, or immunological disorders, performing a primarily protective function. However, in cases of prolonged or excessive inflammation, the development of severe complications and secondary pathologies is possible.

In Ukraine, the primary means of treating inflammation remains synthetic pharmacological drugs, in particular non-steroidal anti-inflammatory drugs (NSAIDs), the effectiveness of which, however, is often