DOI 10.26724/2079-8334-2023-1-83-121-125
UDC 616-001.45-003.6:579.222.4-092

I.A. Lurin[1], V.V. Makarov[2,3], V.V. Nehoduiko[2,3], S.V. Tertyshnyi[4], R.S. Vastyanov[5], S.M. Chobey[6], S.O. Korol[7]
State Scientific Institution "Scientific and Practical Center of Preventive and Clinical Medicine" of the State Administration of Affairs, Kyiv
[1]National Academy of Medical Sciences of Ukraine, Kyiv
[2]Kharkiv National Medical University, Kharkiv
[3]Military Medical Clinical Center of the Northern Region of the Command of Medical Forces, Armed Forces of Ukraine, Kharki
[4]Military Medical Clinical Center of the Southern Region of the Command of Medical Forces, Armed Forces of Ukraine, Odesa
[5]Odesa National Medical University, Odesa
[6]Uzhhorod National University, Uzhhorod
[7] Ukrainian Military Medical Academy, Kyiv

TERMINATION TRANSFORMATION OF THE FRAGMENTARY CAPSULE AFTER A GUNSHOT

e-mail: tertyshnyi.sergey@gmail.com

By the method of Mikel Calvo in the modification of Davydenko S.I. by staining the carboxyl and basic groups of proteins, it was proven that the perversion of the synthesis and maturation of the connective tissue of the periosteal capsule is due to the presence of metal fragments, soot, chemicals and elements. In the capsule around the fragment located in the dermis, in the period from 4 months to 6 years, due to contact with large and small metal fragments, disorganization of collagen occurs with a decrease in the relative number of basic amino groups of proteins and an increase in the relative number of acidic proteins due to carboxyl and hydroxyl groups. Endless destruction of maturing connective tissue fibers by carboxyl groups of proteins and their predominance over basic groups contributes to tissue acidosis and enhances metabolic proteolysis processes, destroying surrounding tissues, preventing tissue healing and leading to final fibrosis and removal.

Key words: gunshot wound, capsule, foreign body, long-term wear.

І.А. Лурін, В.В. Макаров, В.В. Негодуйко, С.В. Тертишний, Р.С. Вастьянов, С.М. Чобей, С.О. Король

ТЕРМІНАЦІЙНА ТРАНСФОРМАЦІЯ ПЕРІОСКОЛОЧНОЇ КАПСУЛИ ПІСЛЯ ВОГНЕПАЛЬНОГО ПОРАНЕННЯ

Методом Mikel Calvo у модифікації Давиденко С.І. шляхом фарбування карбоксильних та основних груп білків доведено збочення синтезу та дозрівання сполучної тканини періосколкової капсули у зв'язку з наявністю металевих фрагментів, кіптяви, хімічних речовин та елементів. У капсулі навколо осколка, що знаходиться в дермі, в термін від 4 місяців до 6 років, через контакт з великими та дрібними металевими фрагментами, відбувається дезорганізація колагену зі зменшенням відносної кількості основних аміногруп білків та збільшенням відносної кількості кислих білків за рахунок карбоксильних та гідроксильних груп. Нескінченне руйнування дозріваючих сполучнотканинних волокон карбоксильними групами білків та їх превалювання над основними групами сприяє тканинному ацидозу і посилює процеси обмінного протеолізу, руйнуючи навколишні тканини, перешкоджаючи загоєнню тканин і приводячи остаточному фіброзуванню та видаленням.

Ключові слова: вогнепальне поранення, капсула, стороннє тіло, тривалі терміни носіння.

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

Connective tissue, being the main type of tissue, is of paramount importance in the life of humans and mammals. It is a supporting tissue for all types of epithelium, muscle and nervous tissue. It plays the role of endomysium, perimisia, endoneurium, perineurium, and special types of connective tissue - bone, cartilage and adipose tissues [2]. Moreover, the connective tissue, normal or with varying degrees of collagen disruption, having a different structure of collagen fibers, replaces all damaged tissues and organs in the body. In such cases, it is called young maturing connective or scar tissue, fibrous, mature, sclerosed and hyalinized [5, 7, 11]. In some cases, such as chronicity of inflammatory processes, in cicatricial changes in organs, its metaplasia occurs [2].

Consequently, the structural and functional properties of the connective tissue determine its quality as a criterion for normal repair and regeneration. The normal structure of fibrous tissue determines the normal functioning of the damaged organ and the quality of human life. The repair and regeneration of tissues after gunshot wounds is of particular importance since in addition to the complex structure of metal

particles, tissues contain particles of tissues, soot, small metal fragments in the form of placers, soil and other physical and chemical contaminants, in contrast to surgical wounds [1, 4, 6].

The Colvo technique, we believe, is undervalued in modern pathomorphology. It is valued for its simplicity and objectivity in identifying the degree of protein dystrophy both in the parenchyma and in the stroma. The terms "limited proteolysis" and "oxidative modification of proteins" are also used to name the ongoing pathological changes [3, 13].

Treatment and healing of gunshot wounds is a very urgent problem in our time of local and global wars and domestic conflicts [8-10, 12].

The purpose of the study was to analyze collagen's structural and functional properties and assess its quality in the soft tissue capsule around a foreign metal body of a gunshot origin at different times after a gunshot wound.

Materials and methods. Histochemical preparations of three military personnel with gunshot wounds, received during the ATO/JFO on the territory of Ukraine, were the material for the study. The authors investigated the state of the integumentary soft tissues and the connective tissue capsule around foreign bodies at various times after its formation, namely, after 4 months, 1.5 years and 6 years after the injury.

The material was obtained during operative interventions. The foreign body was removed together with the capsule. Pieces of the capsule were fixed in 40 % neutral formalin and subjected to paraffin wiring according to the method adopted in the work of pathological laboratories. After paraffin embedding, sections with a thickness of 5–6 μm were made on a sledge microtome MS-2.

There are several histochemical methods for staining connective tissue, allowing us to assess its morphofunctional properties. However, Mikel Calvo (1957) has found that pathological and malignant changes in connective tissue occur in the structure of general proteins [3]. The changes concern, primarily, the ratio of carboxyl and hydroxyl groups of proteins in comparison with amino groups' content, expressed by the R/B ratio. "Red" – red coloration is typical for "acidic proteins", while blue is for "basic proteins" – "Blue".

The authors have found that exceeding the value of the R/B ratio encourages remodelling of connective tissue, increasing its permeability and weakening barrier functions. A decrease indicates the accumulation of "acidic" proteins promoting acidosis, alteration of the parenchyma and stroma of organs, violation of scar density, and prevents wound healing.

Since this method gives such a double result, it is advisable to use it for assessing the prognosis of wound healing and ascertaining the quality of connective tissue.

The essence of the technique is that bromophenol blue, subject to certain staining procedures, stains "acidic" and "basic" proteins differentially. In addition, "basic" proteins are colored light blue and blue, and "acidic" proteins are colored red, yellow and green.

We used staining with bromophenol blue according to the method of Mikel Calvo (1957) modified by S.I. Davydenko (2017) with subsequent quantitative assessment by computer microspectrophotometry. In our research, we have used an Axiostar-plus microscope (Zeiss, Germany) with photographing on a ProgRes C10 plus camera for microscoping analysis. Using computer images of micropreparations and the VideoTest (RF) program, we have calculated the R/B ratio, where R is the intensity of reflected light in the red range, and B is the intensity of reflected light in the blue range. Thus, we determined the optical density of the connective tissue capsule around the fragments (Red/Blue) [3].

Results of the study and their discussion. Using staining with bromophenol blue according to the method of Mikel Calvo (1957) modified by S.I. Davydenko (2017), allowed us to assess anew the histochemical changes in the collagen of the peri-fragmented fibrous capsule in direct contact with a metal fragment. Metallic microparticles were found in the tissue of the fragmented fibrous capsule at different times after the injury. These parts of the main fragment, as well as the fragment itself, are embedded in their additional fibrous capsules, located in the tissues of the main capsule. The micro-fragments, like the main fragment in the histological specimen, were brown.

The study of cases when a connective tissue capsule formed around the shell fragment at different periods shows that when using histochemical staining, bromophenol blue collagen of the wall of the connective tissue capsule stains both blue and red, with areas of purple. Moreover, the both blue and red-purple coloration of collagen fibers can be characteristic of the earlier formed inner layers of the capsule, around micro-fragments in the thickness of the capsule, and for the outer layers, formed later (fig. 1).

This phenomenon evidences that the process of capsule formation is dynamic and does not stop at any time from the moment of damage. Moreover, it may indicate the absence of maturation of the capsule's inner layers in direct contact with the fragment.

Examination of the shell fragment capsule 4 months after the injury by the method of computer microspectrophotometry has shown that the value of the R/B spectrum ratio in the outer areas of the collagen fibers, colored in blue, is 0.87±0.05, in areas colored in red – 1.46±0.08. In areas, visually assessed as purple colored, the R/B ratio is intermediate. That is, "acidic" proteins containing carboxyl and hydroxyl groups affect the composition of the connective tissue capsule, creating conditions for alteration of both the capsule itself and soft tissues, disrupting their regeneration.

In this observation, the inner layer of the fibrous capsule has not only formed in 4 months but also decreased, as is typical for mature connective tissue. The distancing of the metal fragment from the biological material eliminated the mechanical, chemical and toxic effects on the newly formed young, then mature scar tissue and promote its healing. Based on this observation, we can conclude that we must thoroughly free the wound location from all constituent particles in the wound channel. We state this, based on the observation, where a small shell fragment in the thickness of the capsule in direct contact with the connective tissue prevented the maturation of its collagen as a result of physical and toxic effects.

When examining the fibrous capsule of the fragment 6 years after the gunshot wound, we noticed an increase in the total area of the cut colored red (fig. 2).

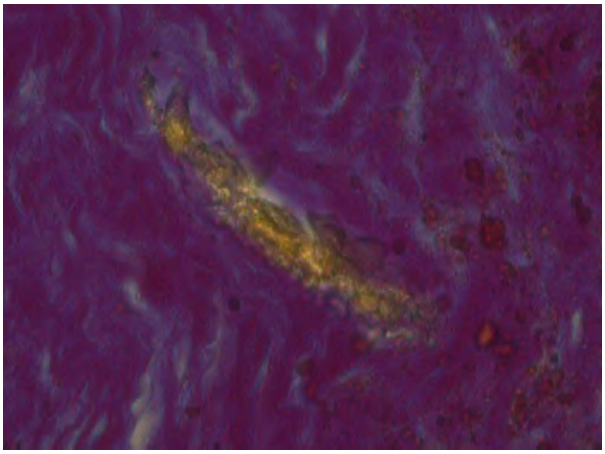

Fig.1. A small metal fragment in the thickness of the capsule 4 months after the injury. The surrounding collagen fibers are colored predominantly red. Staining with bromophenol blue according to Mikel Calvo's method, modified by S.I. Davydenko × 400.

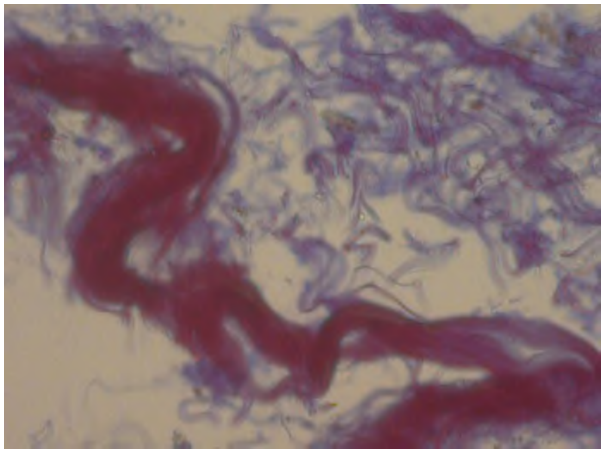

Fig. 2. Tissue of the fragment capsule 6 years after the injury. The inner layer of the capsule has a very compact collagen that turns red. Outside – small collagen fibers dominated by blue coloration. Staining with bromophenol blue according to Mikel Calvo's method, modified by S.I. Davydenko × 100

That is, collagen fibers contain a significant amount of acidic carboxyl and hydroxyl groups. According to the results of microspectrophotometry in the R/B ratio, the optical density of blue fibers is 0.83±0.06 c.u. ($p \geq 0.05$), and more than twice as many red fibers – 1.79±0.07 c. u. ($p \leq 0.01$). When comparing these indicators at different times after the injury, it turns out that the longer the metal fragment is in the tissue, the higher the value of the R/B index is. This can be interpreted as a shift in the acid-base state of the collagen protein towards a more acidic one caused by the long-term negative exposure to tissue contamination of the fragment bed during the capsule formation. That is, the connective tissue has lost its normal relationship between tissue homeostasis and subsequent phases of maturation and healing. According to Mikel Calvo, this occurs by changing the ratio between amino groups and carboxyl groups towards the dominance of carboxyl groups.

Despite the 6-year period of a fragment's residence in soft tissues, toxic effects of metal, soot and chemical substances of the shell fragment have a negative effect on tissues, preventing their complete healing, leading to proteolysis of proteins. As the outer layers of the capsule are removed from the fragment, dominant basic proteins appear and the collagen structure normalizes.

When examining the biomaterial of the fragment capsule from different areas equidistant from it, we revealed a mosaic color pattern. Sections of the capsule, located at the same level as the fragment, could be colored both red and blue.

With computer microspectrophotometry, the authors established that the optical density of the blue collagen areas significantly decreased with longer contact with the fragment, while the optical density of the red collagen areas greatly increased (Table 1).

That is, over time after the injury, collagen accumulates with the dominance of carboxyl groups over amino groups, and the amount of collagen with the amino groups' content decreases. This negative transformation of connective tissue and its main component is an obstacle to wound healing, affecting the limitation of proteolysis.

Table 1

The optical density of different areas of the collagen of the peri-fragment capsule located in the muscle tissue during staining of micropreparations with bromophenol blue (conventional units of optical density)

Term of the wound and the formation of a capsule around the fragment	Areas of collagen capsule blue	Areas of collagen capsule red
4 months	0.40±0.02	0.65±0.03
6 years	0.30±0.02, p≤0.05	0.76±0.04, p≤0.05

Studying the peculiarities of the peri-fragment capsule, staining with bromophenol blue, 1.5 years after the injury of the skin and subcutaneous tissue, (figs. 3, 4) the authors have found that a small capsule fragment, free from any small metal particles, looks dense, compact, stained blue with low R/B ratio (0.69±0.03) and low optical density (0.23±0.01 conventional units of optical density).

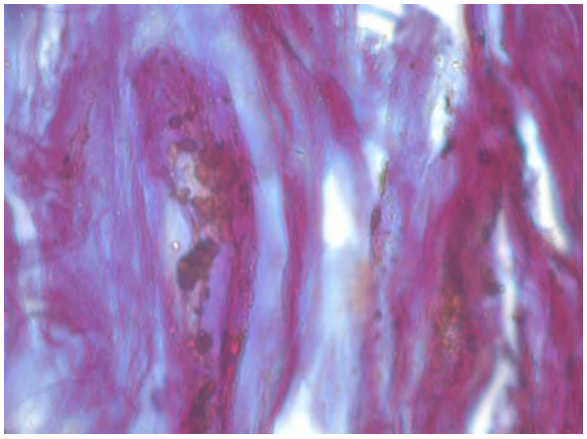

Fig. 3. A fragment capsule in the hypodermis 1.5 years after the injury. Collagen of the fibrous tissue around the metal particles is colored red. Staining with bromophenol blue according to Mikel Calvo's method, modified by S.I.Davydenko × 400.

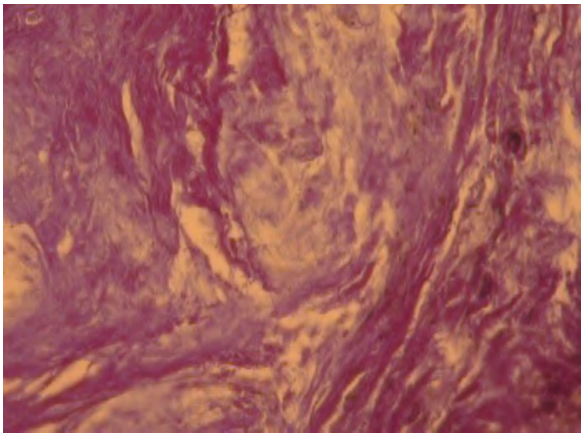

Fig. 4. A fragment capsule in the hypodermis 1.5 years after the injury. Collagen fibers of fibrous tissue with red and blue coloration "alternate". An oval area with young brown collagen fibers is in the center of the photo. Staining with bromophenol blue according to Mikel Calvo's method, modified by S.I.Davydenko × 400.

We can consider such collagen mature, non-dystrophied, complete healing, since the collagen protein has a dominance of amino groups over carboxyl groups in this area.

At the same time, the middle and outer layers of most of the capsule perimeter contain the smallest metal parts, and collagen is colored red around such foci. Wide collagen fibers in the inner layers of the capsule are also red. Such a staining mosaic of the peri-fragment capsule indicates that small metal fragments delay synchronous maturation of the capsule and wound healing.

The mean R/B ratio of collagen fibers colored red is 1.50±0.07. Collagen fibers, colored red, in the capsule wall are interspersed with fibers colored blue. The latter look much thinner, they are arranged chaotically, which indicates their destruction by carboxyl groups. The average R/B ratio of these blue interlayers is 0.83±0.04

Optical density of collagen in peri-fragment fibrous capsule in the blue and red areas also differs (Table 2).

Table 2

The optical density of collagen of the peri-fragment capsule in hypodermis during micropreparations' staining with bromophenol blue (conventional units of optical density)

The term of the wound and formation of a capsule around the fragment	Areas of collagen capsule blue (conventional units of opt.area	Areas of collagen capsule red (conventional units of opt.area
1.5 years	0.83±0.03	1.50±0.06

The presence of brown collagen, containing mainly hydroxyl and carboxyl groups, is evidence not only of the late, newly formed collagen of the capsule but also the destruction of the mature blue collagen of the outer layers of the capsule.

By the method of Mikel Calvo in the modification of Davydenko S.I. [3, 13] by staining the carboxyl and basic groups of proteins, it was proven that the perversion of the synthesis and maturation of the connective tissue of the periosteal capsule is due to the presence of metal fragments, soot, chemicals and elements. In the capsule around the fragment located in the dermis, in the period from 4 months to 6 years, due to contact with large and small metal fragments, disorganization of collagen occurs with a decrease in the relative number of basic amino groups of proteins and an increase in the relative number of

acidic proteins due to carboxyl and hydroxyl groups [1, 2]. Endless destruction of maturing connective tissue fibers by carboxyl groups of proteins and their predominance over basic groups contributes to tissue acidosis and enhances metabolic proteolysis processes, destroying surrounding tissues, preventing tissue healing and leading to final fibrosis and removal [4].

Conclusions

1. During the studied period, from 4 months to 6 years, the authors have found that there is collagen disorganization caused by contact with large and small metal fragments with a decrease in the relative amount of the main amino groups of proteins and an increase in the relative number of acidic proteins due to carboxyl and hydroxyl groups in the capsule around the shell fragment, located in faeces. Interlayers of thinned collagen fibers of blue color indicate even late destruction of mature collagen, possibly after the body has utilized foreign bodies of a firearm origin, containing metals, and soot and having a toxic effect on tissues and the body as a whole.

2. Applying Mikel Calvo's method modified by S.I. Davydenko by staining the carboxyl and basic groups of proteins, the authors have proved for the first-time perverted synthesis and maturation of the connective tissue of the peri-fragmentation capsule due to the presence of metal parts, soot, chemicals and elements.

3. Endless destruction of maturing connective tissue fibers by carboxyl groups of proteins and their prevalence over the main groups promotes tissue acidosis and enhances metabolic proteolysis processes, destroying surrounding tissues, preventing tissue healing and leading to final fibrosis. There is a local, constantly ongoing process of tissue “circulus vitiosus perpetum mobile”, which has no final result. Such a biological process dictates the need to perform the maximum possible rehabilitation of the wound focus and to remove all foreign bodies from the wound.

References

1. Atlas of combat surgical trauma (experience of anti-terrorist operation/joint forces operation). Under the general editorship VI Tsymbalyuk. Kharkiv: Collegium, 2021. 385 p.
2. Barannik MI. Pathomorphological tissue changes on implantation of bioplastic and synthetic materials depending on physical condition. Abstract of the doctoral dissertation. 2014. Novosibirsk. 32 p.
3. Coupland RM, Rothschild MA, Thali MJ. Wound Ballistics: Basics and Applications. Edited by B.P. Kneubuehl. Springer-Verlag Berlin Heidelberg, 2011. 496 p.
4. Davydenko IS. Modification of the histochemical division method into “acid” and “basic” proteins according to Mikel Calvo for the possibility of its application on histological sections, blood smears and preparations-prints. Proceedings of the 98th final conference of the teaching staff of the higher state educational institution of Ukrain "Bukovyna State Medical University", February 20, 2017. Chernivtsi: Medical University. 5–6.
5. Franke A, Bieler D, Friemert B, Schwab R, Kollig E, Güsgen C. The First Aid and Hospital Treatment of Gunshot and Blast Injuries. Dtsch Arztebl Int. 2017. Vol.114, No.14. P. 237–243. DOI://doi.org/10.3238/arztebl.2017.0237.
6. Hill PF, Edwards DP, Bowyer GW. Small fragment wounds: biophysics, pathophysiology and principles of management. J R Army Med Corps. 2001;147(1):41–51. DOI://doi.org/10.1136/jramc-147-01-04. PMID: 11307676.
7. Jarraya M, Hayashi D, de Villiers R, Roemer F, Murakami A, Cossi A, et al. Multimodality Imaging of Foreign Bodies of the Musculoskeletal System. AJR: 203, 2014: 92–102. DOI://doi.org/10.2214/AJR.13.11743.
8. Negoduyko VV, Mykhailusov RM, Yakimova TP. Phases of carrying foreign soft tissue objects of fire origin. Ukrainian Journal of Surgery. Lyman. 2018;2(37). http://dx.doi.org/10.22141/1997-2938.2.37.2018.147848.
9. Soft tissue gunshot wounds (experience of anti-terrorist operation/joint forces operation). Under the general editorship VI Tsymbalyuk. Kharkiv: Collegium, 2020. 400 p.
10. Stefanopoulos PK, Pinialidis DE, Hadjigeorgiou GF, Filippakis KN. Wound ballistics 101:| the mechanisms of soft tissue wounding by bullets. Eur J Trauma Emerg Surg. 2017;43(5):579–586. DOI://doi.org/10.1007/s00068-015-0581-1.
11. Willy C, Hauer T, Huschitt N, Palm HG. «Einsatzchirurgie» - experiences of German military surgeons in Afghanistan. Langenenbecks Arch Surg. 2011; 396 (4): 507–522. DOI://doi.org/10.1007/s00423-011-0760-4.
12. Yongqiang Zh, Dousheng H, Yanning L, Xin M, Kunping W. Peacekeepers suffered combat-related injuries in Mali: a retrospective, descriptive study. BMJ Mil Health. 2020; 166:161–166.
13. Zorenko Y, Singh R. Staining with bromophenol blue as method for studying the neuropil protein of cerebral hemispheres in rats with experimental Alzheimer's type dementia. International Scientific Interdisciplinary Conference, ISIC – 2021: abstract book. KhNMU; 2021: 34–35.

Стаття надійшла 17.02.2022 р.