

STRUCTURAL ORGANIZATION OF RAT TISSUES IN EXPERIMENTAL COMBINED TRAUMA OF THE CHEST AND BOTH THIGHS

*M. Marushchak¹, O. Mialiuk², M. Kaskiv², M. Demjanchuk², I. Krynytska¹

1 – I. HORBACHEVSKY TERNOPIL NATIONAL MEDICAL UNIVERSITY, TERNOPIL, UKRAINE

2 – RIVNE MEDICAL ACADEMY, RIVNE, UKRAINE

Background. In cases of severe trauma, fractures of long bones are the most often combined with trauma of the chest, head, and abdomen, as well as development of hemorrhagic shock. Therefore, it is reasonable to study the combined trauma of the chest and lower extremities in details, as well as the post-traumatic multiple organ dysfunction especially in early manifestation stage.

Objective. The aim of the study was to identify the features of structural organization of the lungs, heart and liver with underlying combined trauma of the chest and both thighs on the 7th day of the post-traumatic period.

Methods. The experimental study involved 22 adult nonlinear white male rats with body mass of 200-210 g, kept on a standard diet at animal facility with food and drinking regimes recommended by the standards for laboratory animals. All animals were divided into 2 groups: the control group (1st, n=10), the experimental group (the 2nd) chest trauma and both thighs trauma, observation for 7 days (n=12). The animals of the experimental group were simulated for right-sided closed pneumothorax with a rib fracture by a trocar under thiopental-sodium anesthesia (40 mg/kg of body weight of the rat, intraperitoneally); it was combined with a fracture of the left and right femurs. Skeletal injury was modeled on each thigh that caused a closed fracture by a single dosed blow with a specially designed device. The blow energy was 0.375 J that corresponds to a severe injury. The associated injury was simulated by successive infliction of both injuries.

Results. Examination of the liver of animals on the 7th day of the experiment revealed a rapid growth of lesions in their parenchyma. The central veins were poorly visible and contained single erythrocytes; the vessels of medium caliber of myocardial stroma were dilated and blood-filled, which was manifested by the development of perivascular oedema. In the lungs of experimental animals, a moderate decrease of interstitial tissue oedema of the interalveolar septa was present, while cellular infiltration of mast cells, macrophages and lymphocytes also decreased significantly.

Conclusions. Multiple organ injuries, which are characterized by structural changes in the liver, heart and lungs in the combined trauma of the thorax and thighs on the 7th day of experiment were evidenced.

KEYWORDS: combined trauma; lungs; heart; liver; morphology.

Introduction

At the present time, the significant increase of multiple and combined traumatic injuries is one of the major health problems in Ukraine as well as in many countries around the world, [1] that leads to polysystemic and multiorgan lesions, which in turn increase mortality rate [2, 3]. The most common injuries and major causes of death are traumatic brain injury [4] and chest injury [5]. According to other research, limb fractures predominate in the structure of polytrauma [6, 7]. In the case of severe trauma, fractures of long bones of the extre-

mities are most often combined with trauma of the chest, head, and abdominal organs, as well as development of hemorrhagic shock [8]. It is established that among patients with trauma, the frequency of polytrauma is 13.7%, and polytrauma with combined multiple fractures of long bones of the lower extremities is 0.5% [9]. The same authors note that more than 70% of chest injuries, traumatic brain injuries, and injuries of the musculoskeletal system (16.7% of patients have fractures of the lower extremities) dominate as for the localization. Therefore, it is reasonable to study the combined trauma of the chest and lower extremities, as well as the post-traumatic multiple organ dysfunction especially in early manifestation stage (on the 7th day).

*Corresponding author: Mariya Marushchak, Professor, I. Horbachevsky Ternopil National Medical University, Ternopil, 46002, Ukraine.
E-mail: marushchak@tdmu.edu.ua

The aim of the study was to define the features of the structural organization of the lungs, heart and liver with underlying combined trauma of the chest and both thighs on the 7th day of the post-traumatic period.

Methods

The experimental study involved 22 adult nonlinear white male rats weighing 200-210 g, which were kept on a standard diet of vivarium with food and drinking regimes at the level recommended by the standards for laboratory animals. All animals were divided into 2 groups: the control group (1st, 10 animals); the experimental group (2nd) – chest trauma and both thighs trauma (CT + 2T), observation during 7 days (12 animals). The animals of the experimental group were simulated right-sided closed pneumothorax with a rib fracture by a trocar under thiopental-sodium anesthesia (40 mg/kg of the body weight of the rat, intraperitoneally); it was combined with a fracture of the left and right femurs. Skeletal injury was modelled on each thigh that caused a closed fracture by applying a single dosed blow by a specially designed device [10]. The blow energy was 0.375 J, which corresponds to a severe injury. The associated injury was simulated by successive infliction of both injuries. While working with animals, the rules of handling experimental animals were followed. Mortality rate of animals was 16.7%.

Samples of heart, liver and lung tissues, under the conditions of simulated associated trauma and correction, were fixed with 1.5% solution of glutaraldehyde in 0.2 M cacodylate buffer (pH 7.2) at $t=4^{\circ}\text{C}$ for 1 h. The samples were washed with cacodylate buffer and additionally fixed with a 2% solution of osmium tetroxide in the same buffer for 1 h ($t=4^{\circ}\text{C}$). The preparations were washed from the fixatives and dehydrated in increasing concentrations of ethyl alcohol (50%, 70%, 90% and 100%). Additionally, they were dehydrated in 2 changes of propylene oxide and placed in epoxy resin epon-812. The sections were obtained using an ultramicrotome UMT-6 and a diamond knife, they were contrasted with 2% uranyl acetate solution for 15 min and additionally with lead citrate according to Reynolds. Sections were observed and photographed using an electron transmission microscope PEM-100.

All animal studies have been approved by the appropriate bioethics committee of I. Horbachevsky Ternopil State Medical University (protocol № 41 of June 1, 2017).

Results

In the most severe period of the maximum probability of complications (from 3 to 10 days) development of respiratory hypoxia, cardiovascular disorders, metabolic acidosis, programmed and induced cell death is possible. During this period the syndrome of multiple organ dysfunction is diagnosed. Therefore, liver, heart and lungs were investigated as target organs in multiple organ dysfunction during associated trauma in 7 days of the experiment. Examination of the liver of animals in 7 days of the experiment revealed a rapid growth of lesions in its parenchyma. The central veins were poorly visible and contained single erythrocytes. The lumens of the sinusoids were practically not visualized, but erythrocytes were traced in their single preserved areas (Fig. 1). The lobular organization of hepatocytes was disturbed, the vast majority of cells did not have a clear structure, their contours were not clear, the size was difficult to visualize. The cytoplasm of hepatocytes of all parts of the lobe was devastated, enlightened, or granular (Fig. 1), which indicated development of severe protein hyaline-droplet and hydropic dystrophy with transition to focal necrosis (Fig. 1). The vast majority of nuclei was crumpled, became smaller, or disappeared, but in some cells they were visualized with loose chromatin, multiple nucleoli. Intercellular connections were largely lost.

Uneven blood supply and focal lympho- and histiocytic perivascular infiltration were observed in the vessels of the portal tracts.

In 7 days of the experiment, the vessels of medium calibre of myocardial stroma were still dilated and blood-filled, which was manifested by development of perivascular oedema. Dilated capillaries and diapedesis of erythrocytes were found in the fibre thickness. Cardiomyocytes were well visualized in the vast majority of visual fields, but focal intracellular myocytolysis and pyknotically altered nuclei were evidenced in some of them, indicating the presence of focal dystrophic-necrotic changes. The perivascular stroma was loosened due to oedema, which also spread to the intercellular spaces, disintegrating the muscle layer (Fig. 2).

In 7 days of the experiment in the lungs of the experimental animals a moderate decrease of interstitial tissue oedema of the interalveolar septa was evidenced, while cellular infiltration of mast cells, macrophages and lymphocytes also decreased significantly. Signs of dystelectasis (partial decline) were still present

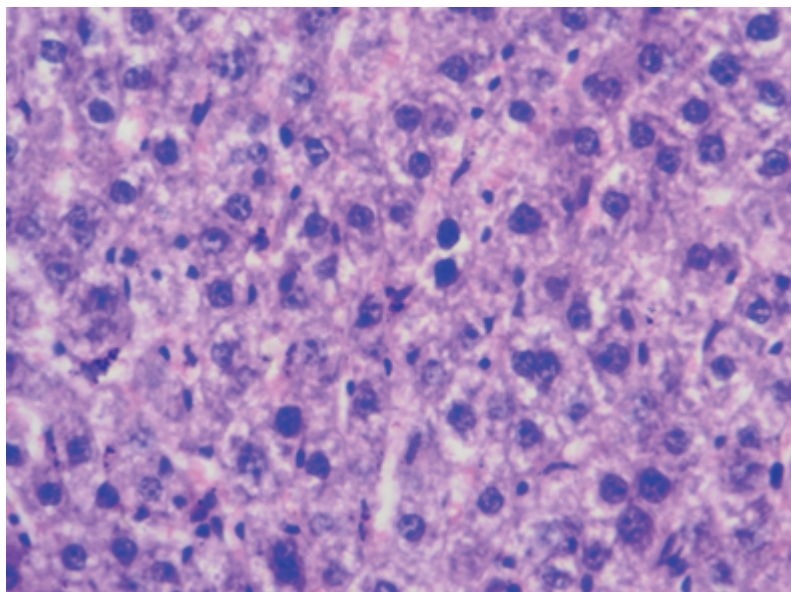


Fig. 1. Structural changes in the liver in cases of simulation of combined trauma in 7 days of the experiment (acute hyaline-drip and hydropic dystrophy with transition to focal necrosis). Hematoxylin and eosin staining. ×200.

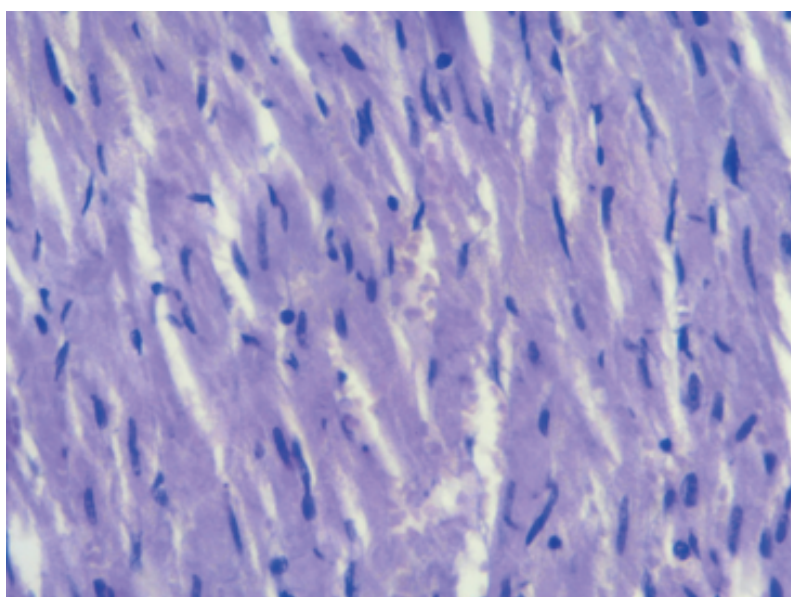
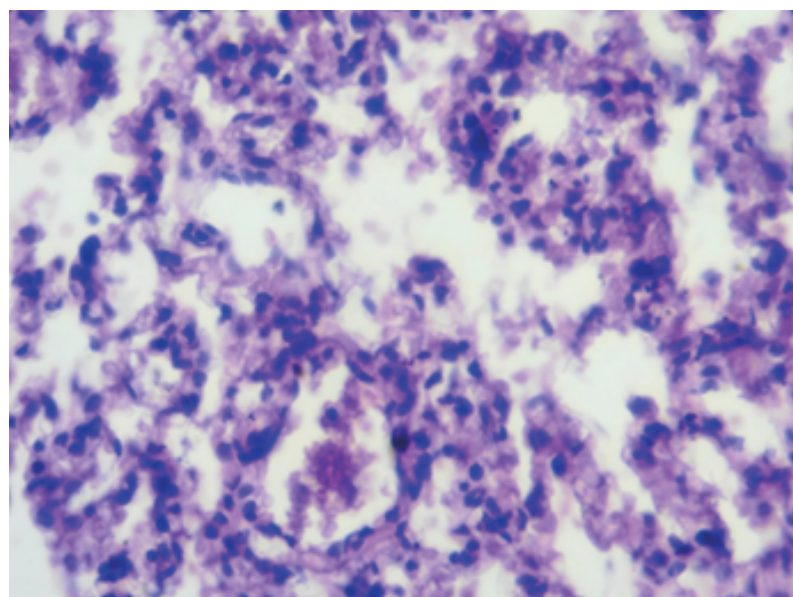


Fig. 2. The structure of cardiomyocytes in the simulation of associated trauma in 7 days of the experiment (pericellular oedema combined with erythrodiapedesis, focal dystrophic changes in cardiomyocytes). Hematoxylin and eosin staining. ×200.

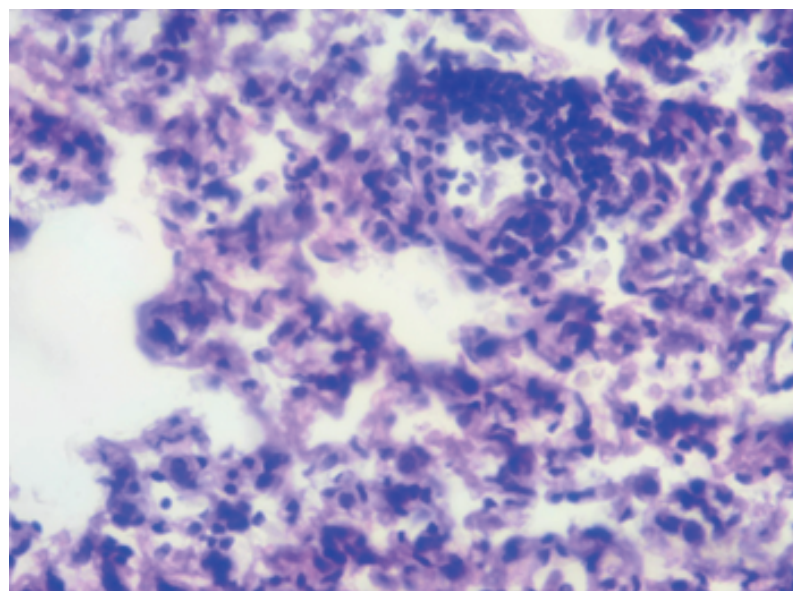
that led to a decrease in the airiness of the lung tissue. Some pneumocytes underwent dystrophic-necrotic changes, and a small amount of exfoliated epithelium and serous exudate appeared in the lumens of the alveoli (Fig. 3). The blood supply to the vessels was still uneven, with a predominance of venous capillary ones. Leukocytes focally accumulated in the perivascular spaces (Fig. 3).

Discussion

The severity of the patient's condition with trauma is largely determined by development of various metabolic disorders in organs and tissues with underlying severe endotoxiosis [11]. These pathological phenomena cause development of deregulatory pathology, one of the manifestations of which is multiorgan



A



B

Fig. 3. Structural changes of the lung in cases of simulation of combined trauma in 7 days of the experiment: A (perivascular accumulation of leukocytes), B (uneven vascular blood supply, dystrophic-necrotic changes of epitheliocytes, slight effusion in the lumen of the alveoli, dystelectasis, focal perivascular leukocyte infiltration). Hematoxylin and eosin staining. $\times 200$.

failure involving lungs, heart and liver into the pathological process.

According to the pathogenesis shortly after the injury the systemic inflammatory response syndrome is established. A number of studies have shown that oxidative stress is crucial in activation of inflammation. From a genetic point of view, oxidative stress modulates inflammation by regulating the activity of certain transcription factors, such as nuclear kappa factor B (NF-kB), signal transducer and activator of transcription 3 signal transducer and

activator of transcription 3 (STAT 3), hypoxia induction factor 1 alpha (HIF-1- alpha), and protein-1 activator (AP-1) [12]. Activation of peroxidation processes is one of the factors destabilizing the membranes. It is established that when hydroxyl radicals influence on diene conjugates of fatty acids, lipid hydroperoxides are formed, which cause conformational disturbances in the cell membranes. The data obtained indicate that the intensity of peroxidation in different tissues of the body depends on the type of injury. Analysis of the scientific

sources shows that oxidative stress in the patients with various injuries develops almost immediately after the initial injury moving from the macroscopic level to the cellular and molecular one [13-15]. Currently, scientists have proven that the maximum of lipoperoxidation coincides with the intensity of the body's response to inflammation [16]. Thus, Kozak DV notes that the intensity of lipoperoxidation due to hip injury and blood loss is fluctuating with a period of development to the 3rd day, a period of temporary well-being in 14 days and a re-increase in 21 days [17]. The results of the study of oxidative processes in the case of combined trauma of the chest and both thighs show that the most intense free-radical processes occur in the lungs on the 7th and 14th days of observation. On the 3rd day of the experiment, the production of mitochondrial superoxide anion radical increases equally in the tissues of the heart and lungs, while in the tissues of the liver this rate increases to a maximum within 3-14 days [18-20]. It has been established that acute hyperproduction of reactive oxygen species by leukocytes in 1 day of the experiment causes disorganization of bioelectrical activity of mitochondrial membranes, which is characterized by a dynamic increase in the ratio of leukocyte cells suspension with reduced transmembrane potential and the ratio of leukocytes with signs of apoptosis with a maximum in 7 days [21].

In cases of extensive metabolic changes in the body tissues of rats with simulated com-

bined trauma, structural changes in the liver, heart and lungs after 1-3 days of the experiment were evidenced. These changes were manifested by severe circulatory disorders, erythrostatics and development of dystrophic-necrotic changes, especially in epithelial structures [22]. According to our study, these structural changes progressed in the lungs, heart and liver up to the 7th day of observation that is comparable to the severity of oxidative stress in the body tissues and apoptotic death of leukocytes.

Conclusions

In cases of combined trauma of the thorax and thighs of the experimental animals, structural changes develop in the liver, heart and lungs on the 7th day of observation. These changes are characterized by severe hyaline-drip and hydropic dystrophy with transition to focal necrosis of liver tissue, pericellular oedema and dystrophic-necrotic changes of pneumocytes.

Conflict of interest

Authors declare no conflict of interest.

Author contributions

Mariya Marushchak, Inna Krynytska – conceptualization, methodology, formal analysis, writing – original draft, writing – reviewing and editing; *Mykhailo Demjanchuk, Oksana Mialiuk* – data curation, investigation, formal analysis; *Marjana Kaskiv* – writing, original draft.

ОСОБЛИВОСТІ СТРУКТУРНОЇ ОРГАНІЗАЦІЇ ТКАНИН ЩУРА ЗА УМОВ ПОЄДНАНОЇ ТРАВМИ ГРУДНОЇ КЛІТКИ ТА ОБОХ СТЕГОН

М. Марущак¹, О. М'ялюк², М. Каськів², М. Демянчук², І. Криницька¹

*1 – ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО
МОЗ УКРАЇНИ, ТЕРНОПІЛЬ, УКРАЇНА*

*2 – КОМУНАЛЬНИЙ ЗАКЛАД ВИЩОЇ ОСВІТИ "РІВНЕНСЬКА МЕДИЧНА АКАДЕМІЯ"
РІВНЕНСЬКОЇ ОБЛАСНОЇ РАДИ, РІВНЕ, УКРАЇНА*

Вступ. За умови тяжкої травми переломи довгих кісток кінцівок найчастіше поєднуються з травмою грудної клітки, голови, органів черевної порожнини, розвитком геморагічного шоку. Тому, обґрунтованим є дослідження поєднаної травми грудної клітки та нижніх кінцівок, а також поліорганність їх ураження, особливо у фазу маніфестації.

Мета. Виявити морфологічні особливості структурної організації легень, серця і печінки на тлі поєднаної травми грудної клітки та обох стегон через 7 діб моделювання травми.

Методи. Експериментальне дослідження виконано на 22 дорослих нелінійних білих щурах-самцях масою 200–210 г, яких утримували на стандартному раціоні віварію з підтриманням харчового і питного режимів на рівні, рекомендованому нормами утримання лабораторних тварин. Усіх тварин

було поділено на 2 групи: контрольну (1-а, 10 тварин), дослідну групу: 2-а – травма грудної клітки й обох стегон (ТГК+2С), спостереження 7 діб (12 тварин). Тваринам дослідної групи під тіопентал-натрієвим наркозом (40 мг/кг маси тіла щура внутрішньочеревно) за допомогою троакара моделювали правобічний закритий пневмоторакс із переломом ребра та поєднували їх з переломом лівої і правої стегнових кісток. Скелетну травму моделювали шляхом нанесення однократного дозованого удару спеціально розробленим пристроєм по кожному стегну, який викликав закритий перелом (Kozak, 2011). Енергія удару становила 0,375 Дж, що відповідало травмі важкого ступеня тяжкості. Поєднану травму моделювали шляхом послідовного нанесення обох ушкоджень.

Результати. Дослідження печінки тварин через 7 діб експерименту встановило різке зростання ураження її паренхіми. Центральні вени проглядалися слабо, містили поодинокі еритроцити, судини середнього калібру стромі міокарда залишалися розширеними та наповненими кров'ю, що проявилось розвитком перивакулярного набряку. У легенях піддослідних тварин спостерігалось помірне зменшення набряку інтерстиціальної тканини міжальвеолярних перегородок, а також значно зменшилась клітинна інфільтрація тучними клітинами, макрофагами та лімфоцитами.

Висновки. При поєднаній травмі грудної клітки та стегон експериментальних тварин структурні зміни в печінці, серці та легенях на 7 добу спостереження, що характеризуються вираженою гіаліново-крапельною та гідропічною дистрофією з переходом у вогнищеві некрози тканин печінки, перичелюлярним набряком, поєднаним з еритродіapedезом, вогнищевими дистрофічними змінами кардіоміоцитів та дистрофічно-некротичними змінами пневмоцитів.

КЛЮЧОВІ СЛОВА: поєднана травма; легені; серце; печінка; морфологія.

Information about the authors

Mariya Marushchak, Professor, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine
<https://orcid.org/0000-0002-1177-412X>, e-mail: marushchak@tdmu.edu.ua

Oksana Mialiuk, Assistant Professor, Rivne Medical Academy, Rivne, Ukraine
<https://orcid.org/0000-0002-5090-6607>, e-mail: oksankamp@ukr.net

Marjana Kaskiv, Assistant Professor, Rivne Medical Academy, Rivne, Ukraine
<https://orcid.org/0000-0002-6914-0867>, e-mail: maryana_kaskiv@ukr.net

Mykhailo Demjanchuk, Assistant Professor, Rivne Medical Academy, Rivne, Ukraine
<https://orcid.org/0000-0001-8729-5144>, e-mail: dmr-rv@ukr.net

Inna Krynytska, Professor, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine
<https://orcid.org/0000-0002-0398-8937>, e-mail: krynytska@tdmu.edu.ua

References

1. Huriev SO, Tanasiienko PV, Satsyk SP. Clinical and epidemiological characteristics of victims of infectious diseases caused by polytraumas of road accidents. *Medicine today and tomorrow*. 2012;1:54.
2. World Health Organization (WHO) Injuries and violence: the facts. (2014). Geneva, Switzerland: WHO. Available from: http://apps.who.int/iris/bitstream/handle/10665/149798/9789241508018_eng.pdf;jsessionid=C29B7C48D83C99FC720F5F318FBF67C8?sequence=1
3. Abio A, Bovet P, Valentin B, Bärnighausen T, Shaikh MA, Posti JP, et al. Changes in mortality related to traumatic brain injuries in the Seychelles from 1989 to 2018. *Front Neurol*. 2021;12:720434. <https://doi.org/10.3389/fneur.2021.720434>.
4. Whizar-Lugo V, Saucedo-Gastelum A, Hernández-Armas A, Garzón-Garnica F, Granados-Gómez M. Chest Trauma: An Overview. *J Anesth Crit Care Open Access*. 2015;3:1. <https://medcraveonline.com/JACCOA/JACCOA-03-00082.pdf>.
5. Chrysou K, Halat G, Hokschi B, Schmid RA, Kocher GJ. Lessons from a large trauma center: impact of blunt chest trauma in polytrauma patients—still a relevant problem? *Scandinavian Journal of Trauma, Resuscitation and Emergency Medicine*. 2017;25(1):42.
6. Probst C, Pape HC, Hildebrand F, Regel G, Mahlke L, Giannoudis P, Krettek C, Grotz MR. 30 years of polytrauma care: An analysis of the change in strategies and results of 4849 cases treated at a single institution. *Injury*. 2009;40(1):77-83. <https://doi.org/10.1016/j.injury.2008.10.004>
7. Banerjee M, Bouillon B, Shafi zadeh S, Paffrath T, Lefering R, Wafaisade A, German Trauma Registry Group. Epidemiology of extremity injuries in multiple trauma patients. *Injury*. 2013;44(8):1015-1021. <https://doi.org/10.1016/j.injury.2012.12.007>
8. Horst K, Simon TP, Pfeifer R, Teuben M, Almahmoud K, Zhi Q, et al. Characterization of blunt

chest trauma in a long-term porcine model of severe multiple trauma. Scientific reports. 2016;6:39659.

<https://doi.org/10.1038/srep39659>

9. Lianskorunskiy VM, Burianov OA, Omelchenko TM, Miasnikov DV, Vakulych MV, Dubrov SO. Analysis of the results of treatment of patients with trauma on the basis of the center of polytrauma. Pain, Anaesthesia & Intensive Care. 2020;4:55-62.

10. Kozak DV. Patent № 63997 Ukraine, Kyiv: Ukraine. 2011.

11. Chernii V. Infusion therapy of traumatic shock. Infusion & Chemotherapy. 2020;3.2:309-311. <https://doi.org/10.32902/2663-0338-2020-3.2-309-311>

12. Chakraborty RK, Burns B. Systemic Inflammatory Response Syndrome. [Updated 2021 Jul 28]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK547669/>

13. Lutsiv II, Hudyma AA, Popovych DV. Influence of cranioskeletal trauma on the dynamics of the enzymatic link of antioxidant protection in the cortical and cerebral layers of the kidney under the conditions of bilateral gonadal removal in the period of late manifestations of traumatic disease. Bulletin of Medical and Biological Research. 2021;2:45-50.

14. Marushchak M, Krynytska I, Petrenko N, Klishch I. The determination of correlation linkages between level of reactive oxygen species, contents of neutrophils and blood gas composition in experimental acute lung injury. Georgian medical news. 2016;253:98-103.

15. Sikirynska DO, Hudyma AA, Pokhodun KA. Features of activation of lipid peroxidation processes in the early period of cranioskeletal trauma complicated by blood loss in rats with different resistance to hypoxia. Medical and Clinical Chemistry. 2020;3: 107-13.

16. Suzuki K, Tominaga T, Ruhee RT, Ma S. Characterization and modulation of systemic inflammatory response to exhaustive exercise in relation to oxidative stress. Antioxidants (Basel). 2020;9(5):401. <https://doi.org/10.3390/antiox9050401>.

17. Kozak DV. Features of indicators of lipid peroxidation in the dynamics of early and late periods of polytrauma. Current issues of transport medicine. 2012;3(29):103-106.

18. Khudobiak MM. Ways to correct free radical oxidation in the early post-traumatic period after a combined injury to the chest and thighs. Medical and clinical chemistry. 2016;18,1(66):10-111.

19. Khudobiak MM, Marushchak MI, Denefil OV. Antioxidant defense system for experimental injuries of the chest and both thighs. Bulletin of Scientific Research. 2016;4(85):134-137.

20. Marushchak MI, Khudobiak MM, Krynytska IYA, Antonyshyn IV. Lipid peroxidation in multiple organ failure caused by associated chest and hip trauma. International Journal of Medicine and Medical Research. 2018;2(2):52-55.

21. Khudobiak MM, Marushchak MI, Holovatiuk LM, Datsko TV. Morphological changes in lungs, heart and liver caused by experimental associated chest and thigh trauma. International Journal of Medicine and Medical Research. 2017;3(1):79-83.

22. Marushchak MI, Khudobiak MM, Habor HH, Mialiuk OP. Mitochondrial mechanisms of apoptosis in combined trauma to the chest and thighs and justification for the use of antioxidants in the experiment. Bulletin of Vinnytsia National Medical University. 2017;1(2):204-210.

Received 28 February 2022; revised 17 May 2022; accepted 15 June 2022.

This is open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.