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ORIGINAL ARTICLE

Features of Metabolism in Chronic Wound Remodelling

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Abstract

Background/Aim: The treatment of chronic wounds continues to be a pressing problem throughout the world. Healing occurs through some evolutionarily conserved biochemical pathways. The mechanisms of development of disorders of reparative regeneration are not fully understood. The work aimed to study the dynamics of changes in metabolic parameters during the healing of chronic wounds.

Methods: Healthy Wistar rats were divided into two groups. The animals of the first group were intact. Chronic wounds were simulated for the animals of the second group. On days 7, 14 and 28 after wound creation, the animals were euthanised. Biochemical parameters such as glucose, total protein, albumin, cholesterol, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were assessed in the blood serum of animals.

Results: It was found that the maximum decrease in glucose and total protein levels in the blood serum of animals in the experimental groups compared to intact animals was observed 2 weeks after surgery: the glucose concentration in rats was 1.7 times lower ($p < 0.001$). The level of albumin in the blood serum of experimental animals compared to intact animals was reduced by 1.5 times after 14 days ($p < 0.001$) and by 1.2 times after 28 days ($p < 0.01$). A week after surgery, the concentration of urea in the blood serum of experimental animals was 1.3 times higher ($p < 0.01$) than in intact rats and by day 28 after surgery, the urea level was 1.4 times higher ($p < 0.001$). The reduction in cholesterol and creatinine levels was not significant. An increase in AST, AST and ALP levels in the blood serum of experimental animals was shown. An increase in the blood serum of animals 7 days after surgery compared to the indicators of intact animals: ALP concentrations by 2.8 times ($p < 0.001$) and ALT concentrations by 1.4 times ($p < 0.001$) was established. The AST level significantly increased 14 days after surgery ($p < 0.05$).

Conclusions: The study of metabolic parameters allows monitoring of the state of the body during the healing process of wounds to correct treatment tactics.

Key words: Wound healing; Metabolism; Rats.

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Introduction

The treatment of chronic wounds continues to be a pressing problem throughout the world. About 6.7 million people worldwide suffer from chronic wounds.[1] Problems in healing chronic wounds are associated with many factors, including the elderly age of patients, concomitant diseases, treatment of wound infections, the influence of emotional and psychological stress, etc.[2] The

degree of involvement of endocrine mechanisms regulating metabolic processes can also serve as a predictor of the chronicity of the process.[3] Metabolism plays an important role in wound healing and defects in metabolic regulation are also implicated in the pathogenesis of chronic wounds.[4] The problem is especially acute in the case of combat trauma, since in combat conditions several factors occur simultaneously that lead to an increased risk of the formation of chronic wounds.

Wound healing is a physiological response to tissue damage. Healing occurs through several evolutionarily conserved biochemical pathways regulated by various cytokines, growth factors and immune cells.[5] Wound healing is divided into three distinct phases: inflammatory, proliferative and remodelling.[6] The inflammatory phase includes haemostasis, chemotaxis of neutrophils and macrophages and increased vascular permeability, which promotes wound cleansing, cell migration, cytokines and reactive oxygen species formations. With the development of chronic inflammation, an imbalance of regulatory mechanisms occurs, characterised by the activity of proinflammatory cytokines and the insufficient effect of their inhibitors and antagonists.[7] Prolongation or delay of the inflammatory phase negatively affects the remaining stages of wound healing.[8] During the proliferative phase, granulation tissue formation, re-epithelialisation and angiogenesis occur. The new blood vessels that are formed help to better meet the metabolic needs of wound healing. During the remodelling phase, granulation tissue is replaced by a permanent scar. Changes that occur during any of these phases may contribute to the development of a chronic wound and interfere with its healing.[9, 10] The other extreme is excessive extracellular matrix formation with disrupted architecture, leading to organ fibrosis (such as hypertrophic scarring of the skin and keloid formation).[11] Skin wound healing involves metabolic reprogramming of glucose, lipids, amino acids and other nutrients that play a vital role in the proliferation, differentiation and migration of various cell types.[12]

The mechanisms of development of disorders of reparative regeneration are not fully understood. There is insufficient information about the features of changes in the basic indicators of metabolic processes during the healing of chronic wounds. The work aimed to study the dynamics of changes in metabolic parameters during the healing of chronic wounds.

Methods

Twenty-four clinically healthy Wistar rats of both sexes aged 8-9 months with a body weight of 220-250 g were used for the study. The animals were divided into two groups and kept under normal vivarium conditions. The first group (n = 6) was intact rats (Int). Eighteen animals of the second group had simulated wounds. The dorsal thoracic area was depilated and prepared for aseptic surgical procedures. Animals were placed under general anaesthesia to create wounds. Each animal was administered tiletamine hydrochloride and zolazepam hydrochloride combination *Zoletil* (Virbac, France) 10 mg/kg intramuscularly (im). Round skin wounds 2 cm in diameter were simulated. The edges of the wound were trimmed with a perpendicular loop-shaped fasciocutaneous suture. On the surface of the wound bottom, the superficial fascia was dissected with mutually perpendicular transverse and longitudinal cuts in the form of a figure measuring 5x5 mm. Then the formed areas were sutured with U-shaped sutures (Figure 1) to create a picture of trophic changes not only in the skin but also in part of the superficial fascia, as well as the underlying tissues. The wound thus obtained represented a model of a chronic wound, reproducing the conditions of local hypoxia and impaired microcirculation.

Figure 1: The induced injury

On days 7 (group Exp7, n = 6), 14 (group Exp14, n = 6) and 28 (group Exp28, n = 6) after wound creation, the animals were euthanised. Blood was

collected from the heart by an open puncture. Biochemical parameters were assessed in animals with wounds and in intact animals. Levels of glucose, total protein, albumin, cholesterol, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in blood serum were determined with the help of reagent kits of *DAC-SpectroMed* (Moldova).

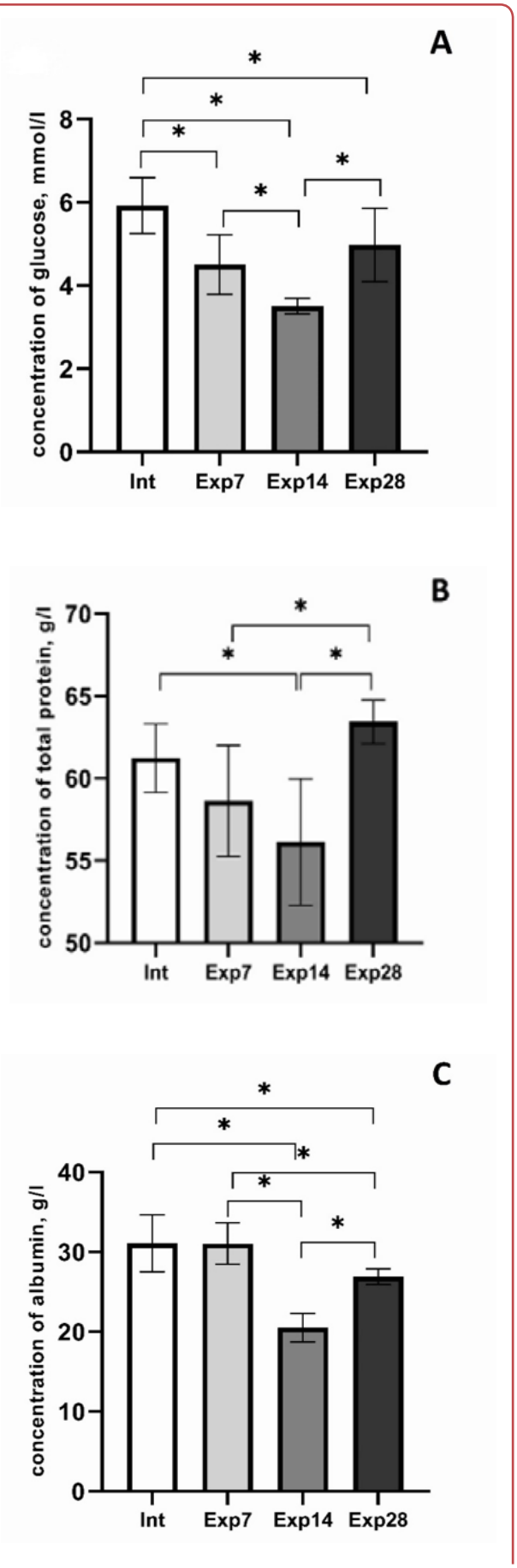
Data analysis

Statistical analysis was performed using *Statistica* 12.0 software (*StatSoft*, USA). The descriptive data were presented as M ± SE, where M is the arithmetic mean and SE is the standard error of the arithmetic mean. The significance of the differences between groups was evaluated using the non-parametric Kruskal–Wallis ANOVA test for independent samples. Differences were considered statistically significant at $p < 0.05$. The relationship between the levels of the studied metabolic parameters was evaluated according to Spearman's rank correlation coefficient (r). Histograms were plotted by *GraphPad Prism* 9 (GraphPad Software, USA).

Results

The concentrations of glucose, total protein, albumin, cholesterol, urea, creatinine, AST, ALT and ALP in the blood serum of animals are presented in Figure 2.

It was found that the level of glucose in the blood serum of animals in the experimental groups was reduced throughout the experiment compared to intact animals (Figure 2A). Thus, two weeks after surgery, the glucose concentration in rats was 1.7 times lower ($p < 0.001$) than the glucose level in intact animals. By the end of the experiment, the glucose level in animals with wounds increased but remained 1.2 times lower ($p < 0.05$) than in intact animals. The concentration of total protein in the blood serum of animals with surgery, compared with that of intact animals, decreased 7 days ($p > 0.05$) and 14 days ($p < 0.01$) after surgery (Figure 2B). However, 28 days after surgery, the total protein levels of the experimental animals returned to the values of intact rats. The level of albumin in the blood serum of experimental animals compared to



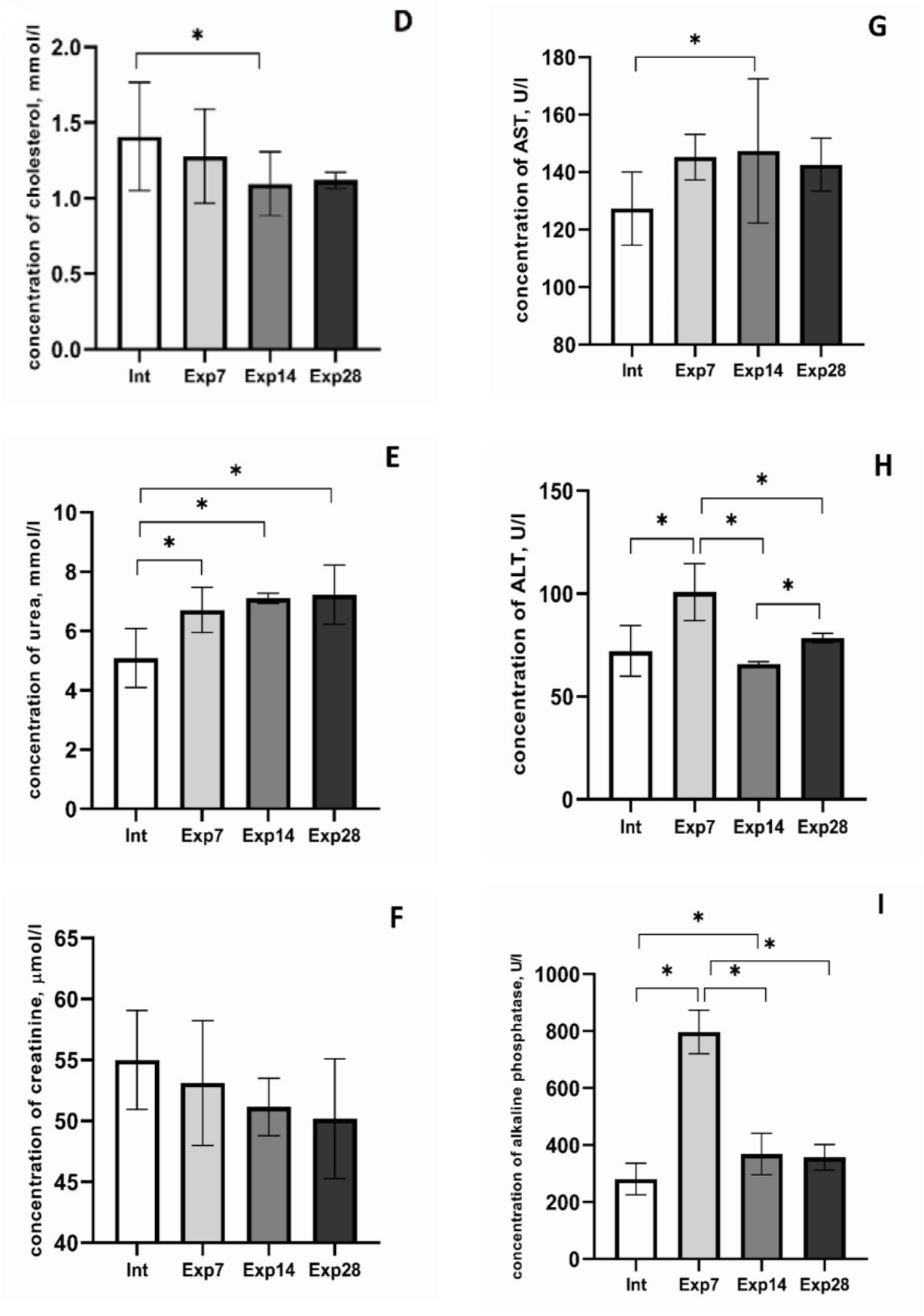


Figure 2: Levels of the studied indicators in the blood serum of animals: (A) glucose, (B) total protein, (C) albumin, (D) cholesterol, (E) urea, (F) creatinine, (G) aspartate aminotransferase (AST), (H) alanine aminotransferase (ALT) and (I) alkaline phosphatase (ALP)

**: $p < 0.05$; The error bars represent the standard error of the arithmetic mean for each indicator (n = 6). Int: intact rats. Exp: experimental groups with simulated wounds euthanised on days 7 (Exp7), 14 (Exp14) and 28 (Exp28) after wound creation.*

intact animals was reduced by 1.5 times after 14 days ($p < 0.001$) and by 1.2 times after 28 days ($p < 0.01$) after surgery (Figure 2C). The levels of cholesterol (Figure 2D) and creatinine (Figure 2F) in the blood serum of animals after surgery, compared with the indicators of intact animals, tended to decrease at all periods of the experiment, although this difference was not statistically significant. The urea concentration in postoperative animals was increased compared with the urea level in intact animals at all experimental times (Figure 2E). Moreover, a week after the operation, the concentration of urea in the blood serum of experimental animals was 1.3 times higher ($p < 0.01$) than in intact rats. And by day 28 after surgery, the urea level was 1.4 times higher ($p < 0.001$). The level of AST (Figure 2G) in the blood serum of animals after surgery had a significant increase compared to the concentration of this enzyme in intact animals only 14 days after surgery ($p < 0.05$). During all other periods of the experiment, the increase in AST levels in experimental animals blood serum was insignificant. The concentration of ALT in the blood serum of animals with surgery, compared with that of intact animals, was increased by 1.4 times 7 days ($p < 0.001$) after surgery (Figure 2H). Fourteen and 28 days after surgery, AST values practically returned to the AST values of intact rats. The concentration of ALP in the blood serum of animals after surgery compared with the indicators of intact animals was 2.8 times increased 7 days ($p < 0.001$) after surgery (Figure 2I). Fourteen and 28 days after surgery, ALP levels decreased, while remaining higher than ALP levels in intact rats.

The relationships between the levels of metabolic parameters in animals of different groups were studied. According to the results of studies in rats of the experimental group, on day 7 after wound induction, positive correlations were noted between the levels of total protein and urea ($r = 0.959$; $p < 0.05$) and between the levels of AST and albumin ($r = 0.935$; $p < 0.05$); a negative correlation was noted between glucose and urea levels ($r = -0.813$; $p < 0.05$). In the experimental group, on the 14 day after surgery, positive correlations were noted between the levels of cholesterol and total protein ($r = 0.846$; $p < 0.05$), between the levels of AST and albumin ($r = 0,865$; $p < 0.05$), between the levels of ALT and albumin ($r = 0.933$; $p < 0.05$); negative correlations were noted between the levels of ALP and total protein ($r = -0.974$; $p < 0.05$) and between the levels of ALP and cholesterol ($r = -0.924$; $p < 0.05$). In the experimental group, on day 28 after wound creation, a negative correlation was established between urea and creatinine concentration ($r = -0.898$; $p < 0.05$).

Discussion

Currently, the research trend is to study the activity of bioactive molecules at various stages of wound healing. Given the high cost of such studies metabolic parameters in the blood serum of animals with chronic wounds were examined to monitor the state of the body, predict healing and adjust treatment tactics using simple and widely available analytes in an outpatient clinic.

Since wound healing is an energy-intensive process, the dynamics of changes in glucose levels during wound healing have been studied (Figure 2A). Glucose concentrations in animals with simulated wounds decreased significantly two weeks after surgery ($p < 0.001$). This may be due to increased glycolysis found in the late stages of acute wound healing and chronic wounds. There is evidence that differences in the increased expression of genes associated with the transport and metabolism of glucose, as well as glycolytic metabolites and enzymes, were more significant in chronic wounds compared to acute ones.[13] Presented chronic wound model replicated hypoxic conditions. The literature has shown an increase in glycolysis and a weakening of mitochondrial function during hypoxia with the development of fibrosis.[14] The hypoglycaemic state of animals, which were observed for 14 days, apparently indicates body exhaustion. Similar results were obtained on day 14 after the hip fracture simulation.[15] On the 28th day of the experiment, glucose concentrations increased, although they did not reach the level of intact animals ($p < 0.05$).

Since a significant decrease in total blood protein levels may be a risk factor for developing postoperative wound healing complications,[16] presented study examined the dynamics of total protein (Figure 2B) and albumin (Figure 2C) levels. A significant decrease in the levels of total protein and albumin ($p < 0.01$), shown in this work on the 14th day of the experiment is associated with the processes of synthesis and breakdown as a result of injury and tissue repair. Thus, the predominance of catabolic processes in

the body of animals was observed. By the end of the study period, an increase in the total protein and albumin concentrations in the blood serum of animals was observed. This can be explained by an increase in the synthesis of amino acids and proteins in the remodelling phase of the wound process and a shift in the balance towards anabolic processes. According to the literature, the total protein and albumin levels in the plasma of rabbits with wound defects did not change significantly on days 3, 7, 14 and 21.[17]

In this study, cholesterol levels were reduced at all stages of wound healing (Figure 2D). In the context of wound healing, lipids act as signalling agents in wound healing and tissue regeneration.[18] The literature has shown that lipid metabolism gene expression is significantly reduced in both acute and chronic wounds, suggesting the downregulation of lipid metabolism.[13]

It is known that the level of urea as the final product of protein metabolism and the main component of residual nitrogen depends on the intensity of its synthesis and excretion. Therefore, in presented study urea concentrations were determined both to assess the health of the liver, where it is synthesised and the kidneys, through which it is excreted (Figure 2E). Urea levels were elevated throughout the wound healing period ($p < 0.005$). It has been shown that shock from extensive trauma can have adverse effects on the harmful kidneys, with outcomes ranging from mild azotaemia to severe kidney damage requiring renal replacement therapy.[19] For example, burn rats at 11 days post-injury showed significant renal and hepatic tissue damage consistent with acute tubular necrosis and multifocal necrosis and changes in blood chemistry (eg, urea and ALT) reflecting deterioration in renal and hepatic function.[20] However, creatinine levels were not elevated. In presented study, creatinine concentrations (Figure 2F) decreased ($p > 0.05$). It can be assumed that this is due to muscle atrophy. This condition is common among patients with persistent critical illness after injury and is associated with increased urea production but decreased creatinine production.[21]

Levels of enzymes such as AST (Figure 2G) and ALT (Figure 2H) were also analysed as markers of tissue destruction.[22] This study showed an increase in the levels of AST and ALT in the blood serum of experimental animals. Seven days after surgery an increase in ALT concentrations in the blood serum of animals was shown compared to the indicators of intact animals. The AST level significantly increased 14 days after surgery.

Presented study also showed an increase in ALP levels in the blood serum of experimental animals 7 days after surgery compared to the values of intact animals (Figure 2I). ALP activity is a marker of acute inflammation because enzyme levels increase in acute wounds but not chronic inflammatory conditions. However, when chronic lesions or scars are healing, an increase in ALP activity is evident.[23]

Increased serum levels of liver biomarkers AST, ALT and ALP may appear to reflect hepatocellular damage.[24] ALT is more specific for hepatocellular injury due to its much higher concentration in liver tissue than in other tissues. In contrast, AST is widely found in a variety of cells, such as hepatocytes, cardiac and skeletal myocytes and erythrocytes.[25] Presented study shows that soft tissue injury affects liver function by causing changes in blood enzyme levels.

Analysis of the relationships between the levels of biochemical parameters showed statistically significant correlations. Regeneration is associated with metabolic processes in different phases of healing. The data obtained are associated with the activation of various signalling pathways that control the process of wound healing. Thus, the results may indicate the essential role of metabolic parameters in the regulation of reparative processes. The wounding process causes metabolic disorders, which are an important factor in the formation of a chronic wound.

Wound treatment must be carried out considering dynamic changes in metabolic parameters. For example, given that during wound healing, glucose metabolism increases and the activity of key enzymes in this process also changes, when treating diabetic wounds it is necessary, first of all, to influence glucose metabolism.[26] The administration of compound protein can accelerate wound healing and improve nutritional status. Because the compound protein accelerates the entire wound healing process, accelerating the transition of wounds from the inflammatory phase to the proliferation phase and entering the remodelling phase earlier.[27] Cholesterol level control is necessary because hypercholesterolaemia delayed wound closure

in mice for several days, likely due to decreased angiogenesis in the wound bed.[28]

Limitations

This study has several limitations. The disadvantages of the chosen model include anatomical differences in the skin of rats and humans. Rodent skin has a layer of *panniculus carnosus*, which causes rapid wound contraction after injury. In contrast, wounds in humans heal through reepithelialisation and granulation tissue formation. Further research is needed to understand whether the regulatory elements of cell metabolism identified in animal models are conserved in the human.[11]

In presented work, it is impossible to statistically reliably determine the role of each metabolic indicator in wound healing. To do this, it is necessary to significantly increase the research volume to conduct factor and cluster analysis. Taking into account the fact that there is insufficient data in the literature to adequately answer this question, there is a plan to carry out this type of research in the future.

Conclusion

The study of metabolic parameters allows monitoring of the state of the body during the healing process of wounds to correct treatment tactics. Proper systemic support with normalisation of metabolic parameters (adequate levels of protein, sugar, etc in the blood serum involved in tissue regeneration) is an important factor in optimising the reparative process. The change in correlations at different phases of the reparative process apparently reflects the activation of various regulatory mechanisms corresponding to a given stage of the reparative process.

Ethics

The experiments were carried out in compliance with the requirements of the Committee on Ethics and Bioethics of Kharkiv Medical Academy of Postgraduate Education (protocol No 2, dated 6 September 2022), consistent with the principles of the European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the Resolutions of the First National Congress on Bioethics (Kyiv, 2001).

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None.

Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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Validation: MK, OL
Formal analysis: MK
Investigation: NB, OL
Resources: RM
Data curation: SP
Writing - original draft: MK, OL
Writing - review and editing: NB, RM
Supervision: RM
Project administration: SP

References

1. Zulkefli N, Che Zahari CNM, Sayuti NH, Kamarudin AA, Saad N, Hamezah HS, et al. Flavonoids as potential wound-healing molecules: emphasis on pathways perspective. Int J Mol Sci. 2023;24(5):4607. doi: 10.3390/ijms24054607.
2. Babenko NM, Litvinova OB, Pavlov SB, Kumechko MV, Komarchuk VV. Problems of healing chronic wounds. Mod Med Technol. 2023;3:66–70. doi: 10.34287/MMT.3(58).2023.10.
3. Pavlov SB, Tamm TI, Komisova TY, Babenko NM, Kumechko MV, Litvinova OB. The nature of changes in endocrine and immune factors at the initial stage of the formation of chronic wounds. Mod Med Technol. 2023;2:34–9. doi: 10.34287/MMT.2(57).2023.6.
4. Hunt M, Torres M, Bachar-Wikström E, Wikström JD. Multifaceted roles of mitochondria in wound healing and chronic wound pathogenesis. Front Cell Dev Biol. 2023;11:1252318. doi: 10.3389/fcell.2023.1252318.
5. Gupta S, Mujawdiya P, Maheshwari G, Sagar S. Dynamic role of oxygen in wound healing: a microbial, immunological, and biochemical perspective. Arch Razi Inst. 2022;77(2):513–23. doi: 10.22092/ARI.2022.357230.2003.
6. Protzman NM, Mao Y, Long D, Sivalenka R, Gosiewska A, Hariri RJ, et al. Placental-derived biomaterials and their application to wound healing: a review. Bioengineering (Basel). 2023;10(7):829. doi: 10.3390/bioengineering10070829.
7. Pavlov SB, Kumechko MV, Litvinova OB, Babenko NM, Goncharova AV. Bone regulatory mechanisms destruction in experimental chronic kidney disease. Fiziol Zh. 2016; 62(3):54–9. doi: 10.15407/fz62.03.054.
8. Pavlov SB, Babenko NM, Kumetchko MV, Litvinova OB, Semko NG, Mikhaylusov RN. The influence of photobiomodulation therapy on chronic wound healing. Rom Rep Phys. 2020;72:609.
9. Almadani YH, Vorstenbosch J, Davison PG, Murphy AM. Wound healing: a comprehensive review. Semin Plast Surg. 2021;35(3):141–4. doi: 10.1055/s-0041-1731791.
10. Pavlov SB, Litvinova OB, Babenko NM. Features of skin wound healing in rats with experimental chronic kidney disease. Regul Mech Biosyst. 2021;12(4):594–8. doi: 10.15421/022181.
11. Eming SA, Murray PJ, Pearce EJ. Metabolic orchestration of the wound healing response. Cell Metab. 2021;33(9):1726–43. doi: 10.1016/j.cmet.2021.07.017.
12. Wang Z, Zhao F, Xu C, Zhang Q, Ren H, Huang X, et al. Metabolic reprogramming in skin wound healing. Burns Trauma. 2024;12:tkad047. doi: 10.1093/burnst/tkad047.
13. Manchanda M, Torres M, Inuossa F, Bansal R, Kumar R, Hunt M, et al. metabolic reprogramming and reliance in human skin wound healing. J Invest Dermatol. 2023;143(10):2039-51.e10. doi: 10.1016/j.jid.2023.02.039.
14. Wang Q, Wang P, Qin Z, Yang X, Pan B, Nie F, et al. Altered glucose metabolism and cell function in keloid fibroblasts under hypoxia. Redox Biol. 2021;38:101815. doi: 10.1016/j.redox.2020.101815.
15. Valilshchykov M, Babalian V, Markina T, Kumetchko M, Boiko L, Romaev S. The enalapril use in arterial hypertension stimulates the reparative processes in fractures of the proximal femur. Indones Biomed J. 2022;14(1):36–44. doi: 10.18585/inabj.v14i1.1736.
16. Neporozhnaya VM. Thrombocytes and some biochemical parameters of blood in patients with different results of healing of facial soft tissues. Bull Dentistry. 2022;118(1):39–42. doi: 10.35220/2078-8916-2022-43-1.7.
17. Handoo N, Parrah JD, Gayas MA, Athar H, Shah SA, Mir MS, et al. Effect of different extracts of R. emodi on hemato-biochemical parameters in rabbit wound healing model. J Pharmacogn Phytochem.2018;7(6):204–10.
18. de Albuquerque PBS, Rodrigues NER, Silva PMDS, de Oliveira WF, Correia MTDS, Coelho LCBB. The use of proteins, lipids, and carbohydrates in the management of wounds. Molecules. 2023;28(4):1580. doi: 10.3390/molecules28041580.
19. Lai WH, Rau CS, Wu SC, Chen YC, Kuo PJ, Hsu SY, et al. Post-traumatic acute kidney injury: a cross-sectional study of trauma patients. Scand J Trauma Resusc Emerg Med. 2016;24(1):136. doi: 10.1186/s13049-016-0330-4.
20. Inoue Y, Yu YM, Kurihara T, Vasilyev A, Ibrahim A, Oklu R, et al. Kidney and liver injuries after major burns in rats are prevented by resolvin D2. Crit Care Med. 2016;44(5):e241–52. doi: 10.1097/CCM.0000000000001397.
21. Haines RW, Zolfaghari P, Wan Y, Pearse RM, Puthucheary Z, Prowle JR. Elevated urea-to-creatinine ratio provides a biochemical signature of muscle catabolism and persistent critical illness after major trauma. Intensive Care Med. 2019;45(12):1718–31. doi: 10.1007/s00134-019-05760-5.
22. Ischenko IO, Tynynyka LN, Kovaliev GA, Yefimova IA, Sandomirsky BP. Effect of cryopreserved cord blood serum on biochemical markers of destruction of tissues. J Exp Clin Med. 2016;1(70):19–25.
23. Krötzsch E, Salgado RM, Caba D, Lichtinger A, Padilla L, Di Silvio M. 162 alkaline phosphatase activity is related to acute inflammation and collagen turnover during acute and chronic wound healing. Wound Repair Regen. 2008;13. doi: 10.1111/j.1067-1927.2005.130216bn.x.

24. Al-Medhtiy MH, Jabbar AA, Shareef SH, Ibrahim IAA, Alzahrani AR, Abdulla MA. Histopathological evaluation of Annona muricata in TAA-induced liver injury in rats. Processes. 2022;10(8):1613. doi: 10.3390/pr10081613.
25. Han JH, Kwak JY, Lee SS, Kim HG, Jeon H, Cha RR. markedly elevated aspartate aminotransferase from non-hepatic causes. J Clin Med. 2022;12(1):310. doi: 10.3390/jcm12010310.
26. Vinaik R, Barayan D, Auger C, Abdullahi A, Jeschke MG. Regulation of glycolysis and the Warburg effect in wound healing. JCI Insight. 2020;5(17):e138949. doi: 10.1172/jci.insight.138949.
27. Wang X, Yu Z, Zhou S, Shen S, Chen W. The effect of a compound protein on wound healing and nutritional status. Evid Based Complement Alternat Med. 2022;2022:4231516. doi: 10.1155/2022/4231516.
28. Bogachkov YY, Chen L, Le Master E, Fancher IS, Zhao Y, Aguilar V, et al. LDL induces cholesterol loading and inhibits endothelial proliferation and angiogenesis in Matrigels: correlation with impaired angiogenesis during wound healing. Am J Physiol Cell Physiol. 2020;318(4):C762-C776. doi: 10.1152/ajpcell.00495.2018.
29. Matsuo K, Matsuzaki S, Roman LD, Klar M, Wright JD. Proposal of an endometrial cancer staging schema with stage-specific incorporation of malignant peritoneal cytology. Am J Obstet Gynecol. 2021 Mar;224(3):319-21. doi: 10.1016/j.ajog.2020.10.045.
30. Kierans AS, Bennett GL, Haghighi M, Rosenkrantz AB. Utility of conventional and diffusion-weighted MRI features in distinguishing benign from malignant endometrial lesions. Eur J Radiol. 2014 Apr;83(4):726-32. doi: 10.1016/j.ejrad.2013.11.030.
31. Mansour TMM, Ahmed YAA-a, Ahmed GAE-R. The usefulness of diffusion-weighted MRI in the differentiation between focal uterine endometrial soft tissue lesions. Egypt J Radiol Nucl Med. 2019;50(1):102. doi: 10.1186/s43055-019-0076-x.
32. Fujii S, Matsusue E, Kigawa J, Sato S, Kanasaki Y, Nakanishi J, et al. Diagnostic accuracy of the apparent diffusion coefficient in differentiating benign from malignant uterine endometrial cavity lesions: initial results. Eur Radiol. 2008 Feb;18(2):384-9. doi: 10.1007/s00330-007-0769-9.
33. Ahmed SA, El Taieb HA, Abotaleb H. Diagnostic performance of sonohysterography and MRI diffusion in benign endometrial lesion characterization. Egypt J Radiol Nucl Med. 2018;49(2):579–89. doi: 10.1016/j.ejrnm.2018.02.010.
34. Gharibvand MM, Ahmadzadeh A, Asadi F, Fazelinejad Z. The diagnostic precision of apparent diffusion coefficient (ADC) in grading of malignant endometrial lesions compared with histopathological findings. J Family Med Prim Care. 2019;8(10):3372–8. doi: 10.4103/jfmpc.jfmpc_142_19.
35. Masroor I, Zeeshan M, Afzal S, Ahmad N, Shafqat G. Diffusion weighted MR imaging (DWI) and ADC values in endometrial carcinoma. J Coll Physicians Surg Pak. 2010 Nov;20(11):709-13. PMID: 21078241.
36. Latif MA, Tantawy MS, Mosaad HS. Diagnostic value of diffusion-weighted imaging (DWI) and diffusion tensor imaging (DTI) in differentiation between normal and abnormally thickened endometrium: prospective study. Egypt J Radiol Nucl Med. 2021;52:107. doi: 10.1186/s43055-021-00487-0.
37. Kececi IS, Nural MS, Aslan K, Danacı M, Kefeli M, Tosun M. Efficacy of diffusion-weighted magnetic resonance imaging in the diagnosis and staging of endometrial tumors. Diagn Interv Imaging. 2016 Feb;97(2):177-86. doi: 10.1016/j.diii.2015.06.013.
38. Beddy P, Moyle P, Kataoka M, Yamamoto AK, Joubert I, Lomas D, et al. Evaluation of depth of myometrial invasion and overall staging in endometrial cancer: comparison of diffusion-weighted and dynamic contrast-enhanced MR imaging. Radiology. 2012 Feb;262(2):530-7. doi: 10.1148/radiol.11110984.
39. Nougaret S, Reinhold C, Alsharif SS, Addley H, Arceneau J, Molinari N, et al. Endometrial cancer: combined MR volumetry and diffusion-weighted imaging for assessment of myometrial and lymphovascular invasion and tumor grade. Radiology. 2015 Sep;276(3):797-808. doi: 10.1148/radiol.15141212.
40. Rechichi G, Galimberti S, Signorelli M, Perego P, Valsecchi MG, Sironi S. Myometrial invasion in endometrial cancer: diagnostic performance of diffusion-weighted MR imaging at 1.5-T. Eur Radiol. 2010 Mar;20(3):754-62. doi: 10.1007/s00330-009-1597-x.
41. Gil RT, Cunha TM, Horta M, Alves I. The added value of diffusion-weighted imaging in the preoperative assessment of endometrial cancer. Radiol Bras. 2019 Jul-Aug;52(4):229-36. doi: 10.1590/0100-3984.2018.0054.