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A new approach to stimulating the wound healing based on *Opisthorchis felineus* proteins

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Motivation and Aim: Skin regeneration is a natural homeostatic process, however, in some cases, chronic non-healing wounds or abnormal scarring develop. This situation is typical for such pathological conditions as deep mechanical damage, severe burns, old age, obesity, diseases of the circulatory system, as well as diabetes mellitus and other autoimmune diseases [1]. Currently, the correction of non-healing wounds is extremely difficult, which dictates the search for new promising therapeutic agents. It should be noted the difficult task of finding drugs that can non-specifically stimulate the processes of skin regeneration, regardless of endo- and/or exogenous triggers of pathological non-healing of wounds. In this regard, the search for new non-specific stimulators of skin regeneration of plant and animal origin seems to be highly relevant. The trematode *Opisthorchis felineus* infects the hepatobiliary system of fish-eating mammals [2]. These parasites have the ability to reduce acute inflammation, and also cause not only damage, but also stimulate the proliferation of bile duct cholangiocytes [3, 4]. Based on this, the aim of the study is to investigate the properties of *O. felineus* proteins as a wound healing agent.

Methods and Algorithms: 80 male mice of the C57Bl/6 line were inflicted with superficial wounds with a diameter of 8 mm. Further, the animals were divided into 8 groups: control (chlorhexidine, 1.5 % methylcellulose, BSA) and experimental (ESP 1, 10, 10 μg without endotoxin; lysate 10 and 50 μg). After applying the solutions, the wound was treated with Luxplast spray plaster (Farmac-zabban, Italy), which created a water-repellent film to protect against contamination and fix the solutions. All groups will receive treatment every 3 days of the experiment with simultaneous detection of the wound area. Animals will be withdrawn from the experiment on days 7 and 10 of treatment. Damaged skin samples were taken for histological examination (formalin and freezing) and for the evaluation of gene expression (RNA-later).

Results: ESP and *O. felineus* lysate proteins significantly increased wound healing in mice ($p < 0.05$). Histological methods showed that accelerated overgrowth was accompanied by a significant decrease in the area of inflammatory infiltration. On 10th day of the experiment, there was no wet crust in the treatment groups, and re-epetelization was detected in the lysate groups. In addition, by day 10, an increase in the number of newly formed CD34+ vessels was diagnosed in the treatment groups, which indicates an improvement in tissue trophism.

According to the results of analysis of the expression of genes-markers of inflammation (Bl_4, Nos2, Arg1), organization of the extracellular matrix (Acta2, Col1α, Col 3,

MMP9, Tgfβ, FGF2, Fn1), the state of the vascular bed (VEGFα) and nervous tissue (NGF, Nestin, NG2) when wounds are treated with lysate and ESP proteins of the trematode *O. felineus*, all of the above processes are completed faster than in animals of the control groups.

Analysis of ESP and lysate of the *O. felineus* proteome revealed not only heme-binding proteins, but also GST, TPX, HDM1 and annexin A2 among the main proteins, which require further individual study as potential participants in wound healing processes.

Conclusion: Thus, preparations based on the trematodes *O. felineus* contribute to faster and better healing of superficial wounds. Apparently, they contain bioactive molecules that can be considered as potential agents for stimulating the regeneration of mammalian tissues and requiring further study.

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References

1. Aitcheson S.M. et al. Skin wound healing: normal macrophage function and macrophage dysfunction in diabetic wounds. *Molecules*. 2021;26(16):4917.
2. Pakharukova M.Y., Mordvinov V.A. The liver fluke *Opisthorchis felineus*: biology, epidemiology and carcinogenic potential. *Trans R Soc Trop Med Hyg*. 2016;110(1):28-36.
3. Maksimova G.A. et al. Effect of *Opisthorchis felineus* infection and dimethylnitrosamine administration on the induction of cholangiocarcinoma in Syrian hamsters. *Parasitol Int.* 2017;66(4):458-463.
4. Kovner A.V. et al. Characteristics of liver fibrosis associated with chronic *Opisthorchis felineus* infection in Syrian hamsters and humans. *Exp Mol Pathol.* 2019;110:104274.