Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Recombinant human growth hormone promotes wound angiogenesis in burned mice through the ERK signaling pathway

Yang Shao[1], Mei Han[1], Guoyue Chen[2], Guodong Song[1]*

[1] Department of Burns and Plastic Surgery &Wound Repair, Jinan Central Hospital, Shandong University, Jinan, Shandong, 250013, China
[2] Department of Medical Imaging, Jinan Central Hospital, Shandong University, Jinan, Shandong, 250013, China

Article Info

Article history:

Received: January 20, 2024
Accepted: April 10, 2024
Published: May 31, 2024

Use your device to scan and read the article online

Abstract

Burns are the most severe type of trauma, and the resulting ischemia and hypoxia damage can promote the dysfunction and even failure of tissues and organs throughout the body, endangering patients' life safety. Recombinant human growth hormone (rhGH) has the functions of promoting protein synthesis to reverse negative nitrogen balance, accelerating wound healing, and improving immune function, which is widely used in the treatment of burns. However, the exact mechanism and pathway of rhGH's action is not yet fully understood. In this study, we observed the wound repair effect of recombinant human growth hormone (rhGH) on burned mice and further analyzed the mechanism of action, which can provide more comprehensive reference opinions for clinical practice. First, by establishing a burn mouse model and and intervening with different doses of rhGH, we found that the wound healing capacity of mice was significantly enhanced and the inflammatory and oxidative stress responses were obviously alleviated, confirming the excellent promotion of wound repair and anti-inflammatory and antioxidant effects of rhGH. Subsequently, we found that the expression of p-ERK1/2/ERK1/2, EGF, TGF-β, and VEGF proteins was elevated in the traumatic tissues of mice after rhGH intervention, suggesting that the pathway of action of rhGH might be related to the activation of ERK pathway to promote the regeneration of traumatic capillaries.

Keywords: Burns, Recombinant human growth hormone, ERK signaling pathway, Angiogenesis, Inflammation response

1. Introduction

Burns are serious type of trauma, and the resulting ischemia and hypoxia damage can promote immune dysfunction of tissues and organs throughout the body, as well as the release of inflammatory neurotransmitters, even leading to functional failure and endangering patients' life safety [1]. Burns rank fourth among the most common traumatic injuries in the human body, with an average of 11 million new burn cases per year [2], of which 180,000 annual global deaths now attributed to burns [3]. Rather than a unified standard for clinical treatment, burns are managed differently, depending on the severity of patients. Severe burns are often accompanied by complications such as immunosuppression, infection, anemia, and severe nutritional losses and deficiencies that require intervention[4, 5]. Growth hormone has been generally recognized by burn surgeons and included in burn treatment guidelines.

Recombinant human growth hormone (rhGH), a protein consisting of 191 amino acid residues [6], can be used as white freeze-dried powder with appropriate excipients or stabilizers, whose basic biological function is to directly metabolize substances and indirectly promote growth. rhGH has the functions of promoting protein synthesis to reverse negative nitrogen balance, accelerating wound healing, and improving immune function, which is widely used in the treatment of burns [7, 8]. Since 2001, rhGH has gradually been emphasized and popularized in the treatment of burns in China. However, there is currently a lack of authoritative reports on the timing, dosage, and mechanism of rhGH use in burn patients, as well as a lack of reliable reference opinions in clinical practice, resulting in some controversy over the use of rhGH.

To confirm the healing effect of rhGH on burn wounds, this study conducts a preliminary analysis of the healing effect of rhGH on burn wounds in mice and the mechanisms of action, which helps confirm the role of rhGH in burn treatment while laying a reliable foundation for the follow-up research on rhGH.

2. Materials and methods

2.1. Animal data

Twenty adult male balb/c mice, specific pathogen-free with a body weight of 15-20 g, were selected for this study.

* Corresponding author.
E-mail address: sgd1232023@163.com (G. Song).
Doi: http://dx.doi.org/10.14715/cmb/2024.70.5.36

The animals were ordered from Taizhou Jianyouda Biomedical Technology Co., Ltd., with the certificate number SYXK (SU) 2022-0029. They were raised in a specific pathogen-free environment (22-25℃, 40-50% humidity, and 12:12-h light: dark cycle) and were allowed to eat and drink freely. This study was approved by Animal Welfare and Ethic Committee of Kangtai Medical Testing Services Hebei Co., Ltd. and strictly adhered to the "3Rs (Replacement, Reduction, and Refinement)" principle of animal experimentation.

2.2. Grouping and modeling

According to the total body surface area (TBSA) = 9.1 × body mass $(W)^{2/3}$, the back depilation area was determined. After fasting for 12 hours, the mice were intraperitoneally injected with ketamine (100 mg/kg) for anesthesia and treated with sodium sulfide (8%) to remove back hair. Following washing with warm water and marking the prescalding area, the back depilation area received a 10% total body surface area (TBSA) and full-thickness burn injury. Immediately after scalding, dexamethasone (80 mg/kg) was injected intraperitoneally, and 0.9% sodium chloride injection (50 mL/kg) was given subcutaneously to prevent shock. Then, 2% iodine tincture was applied to the wound, 8 h/time. The burned mice were then randomized into the control group and groups A, B, and C, with 5 mice in each group. The control mice were injected with 2.00 mL of normal saline under the local wound, while groups A, B, and C were injected with 0.2, 0.4, and 0.6 U/mL of rhGH (Changchun GeneScience Pharmaceutical Co., Ltd.) under the local wound, respectively, once a day, for 14 days.

2.3. Wound healing

Wound photos with scales were taken on the 0th, 3rd, 7th, and 14th days after rhGH intervention, and the wound area was calculated by Image J image analysis software.

2.4. Sample collection

All mice were killed under anesthesia after rhGH intervention for 14 days. The full-thickness skin of the wound was cut off: part of it was added to normal saline to prepare tissue homogenate; some was fixed in paraformaldehyde for the detection of pathological changes of wound surface; and another part was stored at -80℃ for Western blot detection.

2.5. Determination of inflammatory and oxidative stress responses

The supernatant was obtained after centrifugation of tissue homogenate, to measure the levels of tumor necrosis factor-α (TNF-α), and interleukin-1β/6 (Il-1β/6) with enzyme-linked immunosorbent assay (ELISA) kits. All steps followed the corresponding kit instructions. In addition, malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) were measured using a fully automated biochemical analyzer (Myriad BS-2800M).

2.6. Hematoxylin-eosin (HE) staining

The back wound tissue was immobilized in 10% formaldehyde, dehydrated by gradient alcohol, transparentized with xylene, embedded in paraffin, and sliced. After dewaxing and routine HE staining, the histopathological changes of the wound were observed.

2.7. Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end-labelling (TUNEL) staining

The immobilized epithelial tissue was sliced routinely and added with protease K, TUNEL reaction liquid, and converter-POD successively. And the nucleus was stained with DAPI. TUNEL staining was shown by a confocal laser scanning microscope (CLSM). Five visual fields were randomly selected to calculate the apoptosis rate.

2.8. Western blot

Wound tissue protein extraction and concentration determination were made using a radioimmunoprecipitation assay (RIPA) lysis buffer and a bicinchoninic acid (BCA) protein assay reagent kit, respectively. Following sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), the protein samples were semi-dry blotted onto polyvinylidene fluoride (PVDF) membrane, sealed with 5% skimmed milk powder for 2 hours, and incubated overnight at 4℃ with ERK1/2, p-ERK1/2, EGF, TGF-β, VEGF, and β-actin (1:1, 000). A second antibody (1:5, 000) was added the next day for 1 hour of room temperature incubation. After that, the luminous liquid was dripped onto the PVDF membrane for image collection by chemiluminescence gel imaging system and gray value analysis by Image J software.

2.9. Statistical analyses

After running the experiments in triplicates, the results were obtained and presented as ($\chi \pm s$). SPSS25.0 was employed for statistical analyses. Multi-group comparisons were performed using repeated-measures analysis of variance and Bonferroni test, with the presence of statistical significance indicated by $P<0.05$.

3. Results

3.1. Modeling and intervention results

During the study, all four groups of mice survived successfully, with no mice death during burn modeling or rhGH intervention.

3.2. Comparison of wound repair effects

The four groups showed no statistical difference in the burn area on days 0 and 3 ($P>0.05$); however, a reduced burn area in groups A, B, and C versus the control group was observed at 7 and 14 days after intervention ($P<0.05$). The burn area of the four groups began to decrease at 3d compared with 0d and reached the minimum at 14d ($P<0.05$, Figure 1).

3.3. Comparison of inflammatory responses

The detection of inflammatory factors revealed lower levels of TNF-α, IL-1β, and IL-6 in groups A, B, and C compared with the control group ($P<0.05$), with those in group C being the lowest and those in group A being higher versus group B ($P<0.05$, Figure 2).

3.4. Comparison of oxidative stress damage

Compared with the control group, the three groups of mice treated with rhGH showed an increase in SOD and CAT and a decrease in MDA ($P<0.05$). Among them, group C had the highest SOD and CAT, followed by group B ($P<0.05$); while group C had the lowest MDA, followed by group B ($P<0.05$, Figure 3).

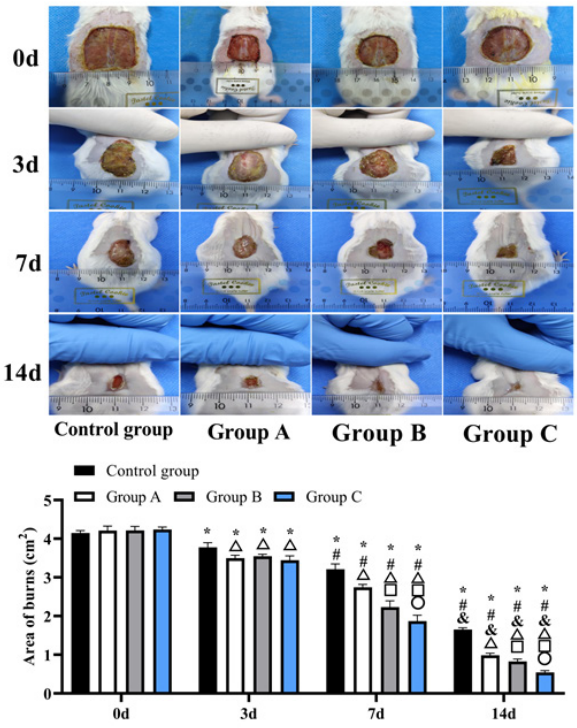

Fig. 1. Comparison of changes in burn area in mice. * denotes P<0.05 compared with 0d, # denotes P<0.05 compared with 3d, & denotes P<0.05 compared with 7d, □ denotes P<0.05 compared with the control group, ☐ denotes P<0.05 compared with group A, ○ denotes P<0.05 compared with group B.

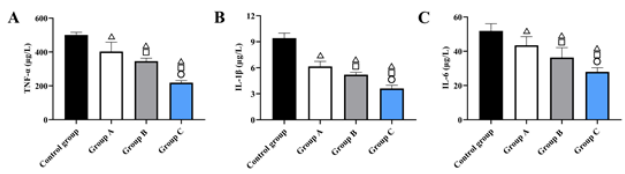

Fig. 2. Comparison of inflammatory responses. A) Comparison of TNF-α, B) Comparison of IL-1β, C) Comparison of IL-6. Δ denotes P<0.05 compared with the control group, □ denotes P<0.05 compared with group A, ○ denotes P<0.05 compared with group B.

3.5. Pathological changes of wound tissue

On the 14th day, the control mice showed more severe inflammatory cell infiltration in the wound tissue, with visible granulation tissue and scarring. In groups A and B, the level of inflammatory cell infiltration in the wound tissue decreased significantly, while the level of epithelialization and collagen formation increased significantly. Group C had no inflammatory cell infiltration and well-grown epithelial cells, with new collagen fibers arranged neatly, which was close to normal skin tissue (Figure 4A). TUNEL staining showed that the apoptosis rate of epithelial cells in the wound tissue of the control group was the highest among the four groups, while that of group C was the lowest (P<0.05, Figure 4B).

3.6. Angiogenesis and ERK pathway expression

After testing, it was found that compared with the control group, EGF, TGF-β, VEGF, and p-ERK1/2/ERK1/2 protein expression in the epithelial tissue of mice in group C was significantly increased (P<0.05, Figure 5).

4. Discussion

Burn wound healing is a complex and orderly biological process controlled by the body. Wound re-epithelization, neovascularization, and continuous granulation tissue formation are the key steps during burn wound healing. In addition, local inflammatory reactions can also occur in the process of wound healing, the significance of which lies in removing damaging elements (including pathogens and other foreign bodies) and necrotic tissues to prevent infection, which is a process that lays the foundation for wound angiogenesis and repair [9]. In this study, we explored the effect of rhGH on burn wound repair, which has important clinical reference significance.

First of all, the burn area of all four groups was found to decrease gradually over time, with a significantly smaller burn area in the three groups of mice treated with rhGH compared with control mice on days 7 and 14, which preliminarily confirms the excellent effect of rhGH on promoting burn wound healing, consistent with the previous

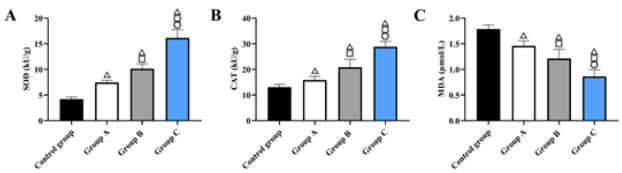

Fig. 3. Comparison of oxidative stress damage. A) Comparison of SOD, B) Comparison of CAT, C) Comparison of MDA. Δ denotes P<0.05 compared with the control group, ☐ denotes P<0.05 compared with group A, ○ denotes P<0.05 compared with group B.

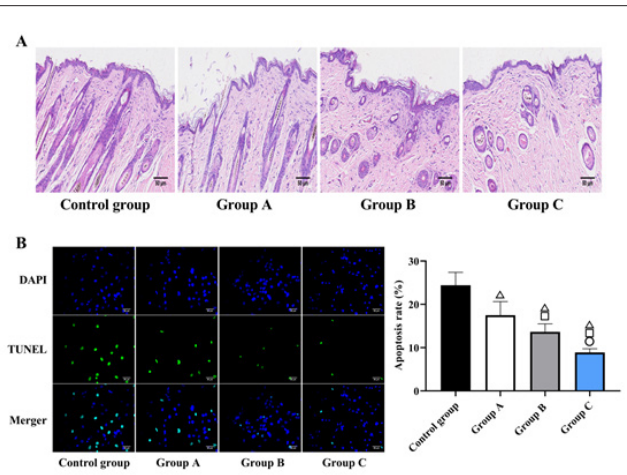

Fig. 4. Pathological changes of wound tissue. A) HE staining of burned tissue (200×), B) TUNEL staining of burned tissue (200×). Δ denotes P<0.05 compared with the control group, ☐ denotes P<0.05 compared with group A, ○ denotes P<0.05 compared with group B.

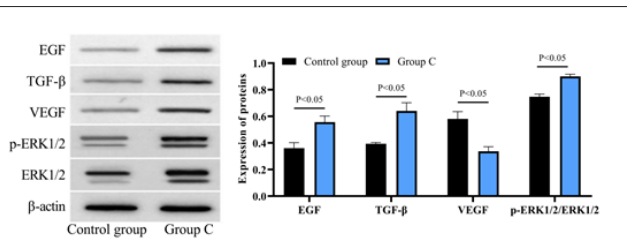

Fig. 5. Angiogenesis and ERK pathway expression. Δ denotes P<0.05 compared with the control group, ☐denotes P<0.05 compared with group A, ○ denotes P<0.05 compared with group B.

research results [10, 11]. Among them, there was no difference in the burn area among the four groups at 0 and 3 days after intervention, which we speculated is related to the periodicity of wound healing. As is well known, the traditional wound healing process can be divided into three phases, namely, the local inflammation phase, cell proliferation and differentiation and granulation tissue formation phase, and tissue remodeling phase, which are interrelated and affect each other over time [12,13]. On days 0 and 3, the trauma of each group of mice is in a period of interaction between the first and second phases, so it was impossible to observe the tendency of rhGH to significantly promote wound shrinkage.

Further observation of inflammation and oxidative stress showed that the levels of inflammation and oxidative products MDA in groups A, B, and C decreased significantly, while antioxidants SOD and CAT increased, indicating that rhGH also has excellent anti-inflammatory and anti-oxidative stress effects. As we all know, skin burns can lead to tissue and cell damage, which in turn promotes the production of massive inflammatory mediators that can react on cells and activate inflammatory signal pathways, leading to the generation of inflammatory mediators in greater quantities, forming a vicious circle, and slowing down the speed and time of wound healing [14]. On the other hand, skin burns can induce tissue edema, tissue hypoxia, imbalance of normal energy metabolism of cells, and the generation of a large number of free radicals, promoting the occurrence of oxidative stress [15]. Excessive oxygen free radicals will damage cells and normal tissues. For example, reactive oxygen species can bind to cell membrane lipids to produce toxic substances such as MDA [16]. Inflammation and oxidative stress are complementary and mutually reinforcing in the pathological changes of burns. RhGH shows excellent anti-inflammatory and anti-oxidative effects, which can also better promote the repair of burn wounds and reduce the risk of complications such as infection. Similarly, we observed a decrease in inflammatory infiltration and epithelial cell apoptosis rate in groups A, B, and C after staining the wound tissues of each group of mice, confirming the excellent effect of rhGH in promoting burn wound repair. Moreover, Cristóbal L et al. also obtained consistent results with ours when exploring the wound repair effect of rhGH on pressure ulcer mice [17], which can support the accuracy of this paper.

Furthermore, the wound repair, anti-inflammatory, and anti-oxidative stress effects increased with the increase of dosage in the three groups of mice treated with rhGH, which also suggests that large doses of rhGH should be selected as far as possible in the future clinical application of rhGH. However, as the pathway through which rhGH promotes wound healing remains elusive, we further selected group C, which has the most significant intervention effect among groups A, B, and C, for in-depth analysis.

Research has shown that ERK is one of the most important signal transduction pathways in cells, playing an important role in inflammatory response, oxidative stress, cytoplasmic function regulation, etc. [18]. Currently, studies have confirmed that the transduction of ERK signaling pathway is closely linked to both inflammatory response and oxidative stress [19]. Meanwhile, Li YH et al. also showed that botulinum toxin type A decreased the proliferation of fibroblasts and prevented overdeposition of ECM through the inhibition of the TGF-β1/Smad and ERK pathways[20]. Therefore, we speculate that the ERK pathway may be also involved in the promotion of burn wound repair by rhGH. After testing, the p-ERK1/2/ERK1/2 protein expression in group C was found to be more significantly increased compared with the control group, confirming that rhGH can activate the expression of phosphorylated proteins of ERK1/2, activate the ERK signaling pathway, reduce inflammatory mediator release, and alleviate inflammation and oxidative stress, thus facilitating the healing of burn wounds. Meanwhile, EGF, TGF-β, and VEGF protein levels in the epithelial tissue of group C were all increased, which fully shows that rhGH can promote capillary angiogenesis during wound healing and promote the synthesis of collagen. GH counld activate ERK pathway[21] and phosphorylation of the ERK1/2 pathway can promote angiogenesis and led to accelerated wound closure, which is consistent with our findings[22]. However, this study only preliminarily explored the activation of the phosphorylated ERK1/2 level by rhGH, but whether rhGH plays a therapeutic role by activation the ERK signaling pathway needs to be further studied.

5. Conclusion

rhGH could effectively promote the wound repair in burned mice and showed excellent anti-inflammatory and anti-oxidative stress injury effects, and its pathway of action might be related to the activation of ERK signaling pathway transduction to promote the regeneration of wound capillaries. In the future, rhGH can serve as an excellent burn treatment scheme to provide patients with a more reliable prognosis.

Conflicts of Interest

The authors report no conflict of interest.

Availability of data and materials

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

Funding

This study was supported by National Clinical Key Specialty; Wang Zhengguo Traumatology Development Foundation "Growth Factor Rejuvenation Program" (NO. SZYZ-TR-15); Jinan Clinical Medical Research Center for Burns/Trauma and Chronic Wounds (NO.201912010); Jinan Science and Technology Innovation Project on Medical and Health (NO.201907080); Shandong Province Medicine Health Science and Technology Development Plan(NO.202004030738).

References

1. Jeschke MG, van Baar ME, Choudhry MA, Chung KK, Gibran NS, Logsetty S (2020) Burn injury. Nat Rev Dis Primers 6 (1): 11. doi: 10.1038/s41572-020-0145-5
2. Radzikowska-Büchner, E., Łopuszyńska, I., Flieger, W., Tobiasz, M., Maciejewski, R., & Flieger, J. (2023). An Overview of Recent Developments in the Management of Burn Injuries. International journal of molecular sciences, 24(22), 16357. doi.org/10.3390/ijms242216357
3. Kankam, H. K. N., Lee, K. C., Sardeli, A. V., Dretzke, J., Lord, J. M., & Moiemen, N. (2022). Acute burn injuries associated with long-term mortality: a systematic review and meta-analysis. Burns : journal of the International Society for Burn Injuries,

48(8), 1783–1793. doi.org/10.1016/j.burns.2022.06.009
4. Palmieri TL (2019) Burn injury and blood transfusion. Curr Opin Anaesthesiol 32 (2): 247-251. doi: 10.1097/ACO.0000000000000701
5. Wischmeyer PE (2019) Glutamine in Burn Injury. Nutr Clin Pract 34 (5): 681-687. doi: 10.1002/ncp.10362
6. Wang F, Qiu L (2013) Advancement in the research of growth hormone in treating pediatric burn patients. Zhonghua Shao Shang Za Zhi 29 (1): 18-21. doi: 10.3760/cma.j.issn.1009-2587.2013.01.006
7. Tian FY, Wu B, Xu T, Jiang XH (2017) Systematic evaluation on effectiveness and safety of recombinant human growth hormone in treating adult patients with severe burn. Zhonghua Shao Shang Za Zhi 33 (9): 568-573. doi: 10.3760/cma.j.issn.1009-2587.2017.09.009
8. Breederveld RS, Tuinebreijer WE (2014) Recombinant human growth hormone for treating burns and donor sites. Cochrane Database Syst Rev 2014 (9): CD008990. doi: 10.1002/14651858.CD008990.pub3
9. Landén, N. X., Li, D., & Ståhle, M. (2016). Transition from inflammation to proliferation: a critical step during wound healing. Cellular and molecular life sciences : CMLS, 73(20), 3861–3885. doi.org/10.1007/s00018-016-2268-0
10. Gilpin, D. A., Barrow, R. E., Rutan, R. L., Broemeling, L., & Herndon, D. N. (1994). Recombinant human growth hormone accelerates wound healing in children with large cutaneous burns. Annals of surgery, 220(1), 19–24. doi.org/10.1097/00000658-199407000-00004
11. Breederveld, R. S., & Tuinebreijer, W. E. (2014). Recombinant human growth hormone for treating burns and donor sites. The Cochrane database of systematic reviews, 2014(9), CD008990. doi.org/10.1002/14651858.CD008990.pub3
12. Sorg, H., Tilkorn, D. J., Hager, S., Hauser, J., & Mirastschijski, U. (2017). Skin Wound Healing: An Update on the Current Knowledge and Concepts. European surgical research. Europaische chirurgische Forschung. Recherches chirurgicales europeennes, 58(1-2), 81–94. doi.org/10.1159/000454919
13. Singer A. J. (2022). Healing Mechanisms in Cutaneous Wounds: Tipping the Balance. Tissue engineering. Part B, Reviews, 28(5), 1151–1167. doi.org/10.1089/ten.TEB.2021.0114
14. Son, B., Lee, S., Kim, H., Kang, H., Kim, J., Youn, H., Nam, S. Y., & Youn, B. (2019). Low dose radiation attenuates inflammation and promotes wound healing in a mouse burn model. Journal of dermatological science, 96(2), 81–89. doi.org/10.1016/j.jdermsci.2019.10.004
15. Parihar, A., Parihar, M. S., Milner, S., & Bhat, S. (2008). Oxidative stress and anti-oxidative mobilization in burn injury. Burns : journal of the International Society for Burn Injuries, 34(1), 6–17. doi.org/10.1016/j.burns.2007.04.009
16. Josh, F., Soekamto, T. H., Adriani, J. R., Jonatan, B., Mizuno, H., & Faruk, M. (2021). The Combination of Stromal Vascular Fraction Cells and Platelet-Rich Plasma Reduces Malondialdehyde and Nitric Oxide Levels in Deep Dermal Burn Injury. Journal of inflammation research, 14, 3049–3061. doi.org/10.2147/JIR.S318055
17. Cristóbal, L., de Los Reyes, N., Ortega, M. A., Álvarez-Mon, M., García-Honduvilla, N., Buján, J., & Maldonado, A. A. (2019). Local Growth Hormone Therapy for Pressure Ulcer Healing on a Human Skin Mouse Model. International journal of molecular sciences, 20(17), 4157. doi.org/10.3390/ijms20174157
18. Guo YJ, Pan WW, Liu SB, Shen ZF, Xu Y, Hu LL (2020) ERK/MAPK signalling pathway and tumorigenesis. Exp Ther Med 19 (3): 1997-2007. doi: 10.3892/etm.2020.8454
19. Liu, P., & Pan, Q. (2022). Butein Inhibits Oxidative Stress Injury in Rats with Chronic Heart Failure via ERK/Nrf2 Signaling. Cardiovascular therapeutics, 2022, 8684014. doi.org/10.1155/2022/8684014
20. Li YH, Yang J, Zheng Z, Hu DH, Wang ZD. Botulinum toxin type A attenuates hypertrophic scar formation via the inhibition of TGF-β1/Smad and ERK pathways. J Cosmet Dermatol. 2021;20(5):1374-1380. doi:10.1111/jocd.13842
21. Frank S. J. (2020). Classical and novel GH receptor signaling pathways. Molecular and cellular endocrinology, 518, 110999. doi.org/10.1016/j.mce.2020.110999
22. Zhao, Y., Wang, X., Yang, S., Song, X., Sun, N., Chen, C., Zhang, Y., Yao, D., Huang, J., Wang, J., Zhang, Y., & Yang, B. (2020). Kanglexin accelerates diabetic wound healing by promoting angiogenesis via FGFR1/ERK signaling. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 132, 110933. doi.org/10.1016/j.biopha.2020.110933