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Propranolol regulates ERK1/2 signaling pathway and promotes chronic wound healing in diabetic rats

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Abstract. – OBJECTIVE: This study aimed to investigate if propranolol could regulate ERK1/2 signaling pathway and promote chronic wound healing in diabetic rats.

MATERIALS AND METHODS: Twenty-two rats were used to establish a diabetic chronic wound animal model. They were randomly separated into two groups: the propranolol group and the control group. The propranolol group was treated with propranolol ointment and the control group was treated with propranolol matrix cream to cover the wound surface. The expression of the p-ERK1/2 protein was detected by the Western Blot. RT-qPCR was used to detect the expression of VEGF. The concentrations of IL-6 and TNF-α were detected by ELISA.

RESULTS: The body weight of rats was significantly reduced after type 2 diabetes mellitus modeling. The healing rate of rats in the control group was significantly lower than that in the propranolol group ($p<0.05$). There was a significant increase in the expression of the *p*-ERK1/2 protein in the wound tissue of the propranolol group compared with that in the control group, except for the 11th day ($p<0.05$). The relative expression of Vascular Endothelial Growth Factor (VEGF) in the propranolol group was significantly higher than that in the control group on the 2nd day ($p<0.05$), while the relative expression of VEGF in the propranolol group was significantly increased on the 11th day after modeling ($p<0.05$). On the 20th day, the expressions of IL-6 and TNF-α in the propranolol group were significantly higher than those in the control group, and there were significant differences ($p<0.05$). It was found that the IL-6 and TNF-α expressions in the propranolol group reached the peak on the 11th day and then gradually decreased ($p<0.05$).

CONCLUSIONS: The results indicated that propranolol can accelerate the healing of diabetic wounds by regulating the expression of VEGF by phosphorylation of ERK1/2 protein, thus promoting chronic wound healing in diabetes.

Key Words:
Propranolol, ERK1/2 signaling pathway, Chronic wound surface, Healing.

Introduction

Wound surface refers to the damage caused by external factors (avulsion, abrasion) or internal factors (nutrition, hypoxia, infection) in human skin tissue. The clinical manifestations are often the loss of skin tissue or the incomplete morphological function of skin tissue at the injured site[1]. If patient's wound healing time is more than 1 month, it will become a chronic refractory wound which will require long-term treatment and high cost, seriously affecting patients' quality of life and becoming a heavy burden[2]. As a chronic metabolic disease, diabetes mellitus is closely related to people's life and exercise habits. In clinical practice, the main clinical manifestations are polydipsia and polyphagia[3]. In a 2017 statistical report[4] in Atlanta, Georgia, there were 23.1 million people with diabetes in the United States in 2015, accounting for 7.2% of the total US population, and more than 253,000 cases of diabetes deaths. In China, there are more than 100 million people with diabetes, the highest rate in the world[5]. With the increase of diabetic patients, the incidence of chronic and difficult wounds in diabetes is gradually increasing. Therefore, how to promote the healing of diabetic wounds and explore the wound healing mechanism of diabetic patients are urgent problems to be solved.

There are abundant sympathetic nerve fibers in the skin and subcutaneous tissues. A previous study[6] has shown that, after stimulating the sympa-

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thetic nerves, a large amount of catecholamine will be released to act on the adrenergic receptors on the cell membrane of target organs, causing some biological effects. According to some researches, β-adrenalin, an important component of adrenergic receptors, plays an important role in a variety of skin diseases and pathological processes. It regulates the expression of β-adrenalin and regulates the sympathetic nerve involvement in wound healing[7]. The propranolol, a β-adrenergic blocker, inhibits sympathetic excitation and catecholamine action[8]. The extracellular regulated kinase (ERK), mainly ERK1 and ERK2, is an important pathway for signal transduction. Ying et al[9] have shown that p-ERK1/2 pathway plays an important role in cell proliferation and angiogenesis.

Therefore, this study explores the effect of the propranolol on p-ERK1/2 signaling on chronic wound healing in diabetic rats for clinical reference.

Materials and Methods

Animal Source

A total of 42 SD rats, 8-10 weeks old, with a weight of 170±20 g (Beijing Weitong Lihua Experimental Animal Technology Co., Ltd., Beijing, China, 101) were selected. All rats were purchased by the Animal Experiment Center of Wuwei People's Hospital. The temperature was 21±2°C, and the relative humidity was 40-70%. The rats were kept in separate cages in 12 h during the day and 12 h during the night. The study was approved by the Ethics Committee of Wuwei People's Hospital, China.

Main Reagents and Materials

The main reagents used were: PCR kit TransScript Green Two-Step qRT-PCR SuperMix (TransGen Biotech, Beijing, China, AQ201-01), TRIzol extraction reagent, PierceTM BCA Protein Assay Kit, RIPA Lysis and Extraction Buffer (Thermo Fisher Scientific Co., Ltd., Shanghai, China, 16096020, 23225, 89901), p-ERK1/2 monoclonal antibody, GAPDH, horseradish peroxidase-labeled goat anti-rat IgG (R&D Systems, Minneapolis, MN, USA, MAB1018, 2275-PC-100, HAF005), Vascular Endothelial Growth Factor (VEGF). The primers were designed and synthesized by GenePharma (Shanghai, China) (Table I). IL-6, TNF-α ELISA kit (PI326, PT512) was purchased by Shanghai Beyotime Biotechnology Co., Ltd. (Shanghai, China), while the streptozotocin (S0130) was purchased by Sigma-Aldrich (St. Louis, MO, USA)

Animal Model Establishment

The model was established after one week of breeding. The type 2 diabetes model was established based on the method of Zhang et al[10] as follows: all rats were fed with a high-fat diet; fasted for 12 hours, with free access to drinking water. 1% streptozotocin made with citric acid (pH 4.5, 0.1 mol/L) was intravenously injected in tails. The rat blood glucose, fasting blood glucose, and glucose tolerance during the experiment were randomly measured. The modeling period was 4 weeks. The modeling success criteria were: 12 h after fasting, the blood glucose peak was 16.7 mol/L with 50% glucose solution, and 11.1 mol/L for 120 min. The body weight of the rats decreased significantly by >50 g. Wound establishment: after the successful establishment of the SD rat type 2 diabetes model, the skin was prepared one day before the modeling, and 100 mg/kg sodium pentobarbital hydrochloride (0.6%) was intraperitoneally injected for anesthesia, and routinely disinfected. The upper middle of the lumbar *vertebrae* was established 2 cm x 2 cm wound surface. Under sterile conditions, we surgically cut the skin along the direction of the spine to establish a defect open wound model. The rat wounds were sterilized by dry gauze, fixed, and molded with penicillin ($4*10^6$U) to prevent infection. After the successful modeling, 42 rats were randomly separated into the control group (n=21) and the propranolol group (n=21). The rats in the propranolol group were administered with propranolol ointment (propranolol content 1%). An appropriate amount of ointment was applied directly to the wound (to completely cover the wound), and the outer layer was covered with sterile gauze, and fixed without affecting the

Table I. Primer sequence.

Gene	Upstream primer	Downstream primer
VEGF	5'-CTACCTCCACCATGCCAAGT-3'	5'-GCTCTGGAACCACCTGTAGA-3'
β-actin	5'-TGACGTGGACATCCGCAAAG-3'	5'-CTGGAAGGTGGACAGCGAGG-3'

normal activities of the rats. The rats in the control group used propranolol matrix cream (propranolol content 0%, the rest of the ingredients were the same as propranolol ointment). The rest of the procedure was consistent with the propranolol group. After completion, the rats were kept in separate cages to avoid biting, and injected with penicillin (sterile 0.9% NaCl, 80,000 units/mouse, 0.1 ml). After modeling, 7 rats were randomly selected on the 2nd, 11th, and 20th days to observe the wound healing condition of the rats [healing rate = (original area of wound - unhealed wound surface) *original area of wound *100%], and the tail tip blood was collected and centrifuged for serum. Then, the rats were sacrificed by anesthesia, and the wound tissue of the rats was collected.

PCR Detection

The total RNA was extracted from the collected rat wound tissue using TRIzol extraction reagent, and the purity, concentration, and integrity of total RNA were detected by ultraviolet spectrophotometer and agarose gel electrophoresis. The total RNA was reversely transcribed using the reverse transcription kit (included in the PCR kit), and the procedure was carried out in strict accordance with the manufacturer's kit. The reversely transcribed cDNA was collected for PCR amplification using TransScript Green Two-Step qRT-PCR SuperMix. The reaction system was: 1 μL cDNA template of VEGF reaction system, 0.4 μL of upstream primers, 0.4 μL of downstream primers, 10 μL of 2×TransStart® Tip Green qPCR SuperMix, 0.4 μL of Passive Reference Dye (50×), and Nuclease-free Water was added to 20 μL. The VEGF reaction conditions were: pre-denaturation at 94°C for 30 s, denaturation at 94°C for 5 s, annealing at 60°C for 15 s, and extension at 72°C for 10 s. Three replicate wells were set for each sample, and the experiment was performed three times. β-actin was used as an internal reference, and the data were analyzed using the $2^{-\Delta ct}$ method.

WB Detection

The collected wound tissue was extracted by RIPA lysis method, and the protein concentration was determined by BCA method. The protein concentration was adjusted to 4 μg/μL and separated by 12% SDS-PAGE electrophoresis. After ionization, the membrane was transferred to polyvinylidene difluoride (PVDF) membrane, and the Ponceau red working solution was added for dyeing; it was immersed in PBST for 5 min and blocked with 5% skim milk powder for 2 h; then, primary antibody was added (1:1000) and blocked at 4°C overnight. The primary antibody was removed by washing, and the horseradish peroxidase-labeled goat anti-mouse secondary antibody (1:5000) was added, incubated at 37°C for 1 h, and rinsed 3 times with TBST for 5 min each time. A filter paper was used to absorb the excess liquid on the film and developed in a dark room after ECL. The protein bands were scanned, and the gray values were analyzed in the Quantity One software. The relative expression level of the protein = the gray value of the target protein band / the gray value of the GAPDH protein band.

ELISA Detection

The rat serum was collected, and 50 μL of different standard solutions were added to the blank wells; 50 μL of the antibody was added to 50 μL of distilled water in the blank control wells; 40 μL of the sample was added to the remaining microwells, and then, 10 μL of the biotin-labeled antibody was added. Subsequently, the plate was incubated at 37°C for 30 min. When we washed the plate, the washing solution of each well was ensured to be full without overflow for 30 seconds, was discarded, and patted dry for 5 times. 50 μL of the enzyme standard solution was added to each well and sealed again. The plate was incubated at 37°C for 60 min, and the plate was washed again 5 times. For the last time, the plate was thoroughly patted with absorbent paper, and 100 μL/well of horseradish peroxidase was added, then the plate was sealed. The plate was incubated at 37°C for 15 min in the dark, and 100 μL/well chromogenic substrate TMB was added and incubated at room temperature for 20 min in the dark. Finally, 50 μL/well of the stop solution was added, and the microplate reader was used for detection within 15 min to determine the maximum absorption wavelength at 450 nm. 3 sets of duplicate wells were set, and the experiment was repeated for 3 times.

Statistical Analysis

In this study, the collected data were statistically analyzed using the SPSS 20.0 software package (IBM Corp., Armonk, NY, USA), and the data were plotted using GraphPad Prism 7 (GraphPad Software, La Jolla, CA, USA). The measurement data were expressed as mean±standard deviation (mean±SD). The paired *t*-test was

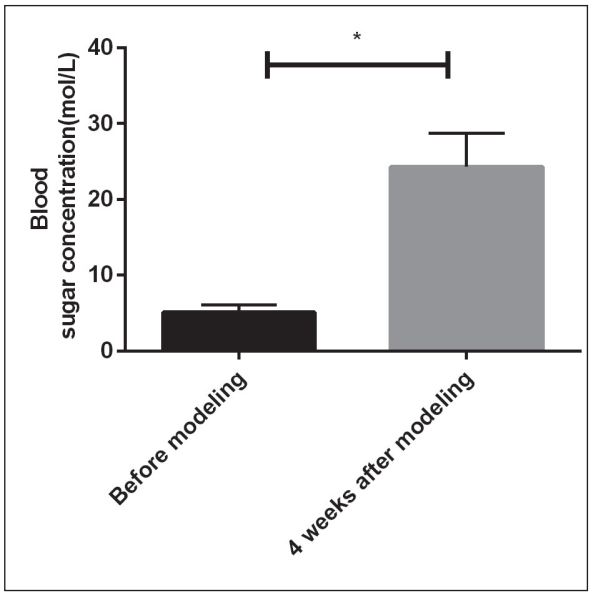

Figure 1. Changes of blood glucose before and after streptozotocin administration in rats. The blood glucose changes before and after streptozotocin in rats were analyzed by paired t-test. It was found that the blood glucose of rats was significantly higher after use than that before use. *indicated a difference between the two groups (p<0.05).

used for the comparison before and after the experiment within the group. The variance analysis was used for the comparison between the groups. The LSD t-test was used after the variance analysis. p<0.05 indicated that there was a statistical difference between the two groups.

Results

General Condition of Rats

After the modeling of type 2 diabetes in the two groups, the body weight was significantly reduced, and the movement response was slow, with symptoms of polydipsia and polyphagia. The comparison of blood glucose in all rats before and after the experiment using streptozotocin showed that the blood glucose level of rats after using streptozotocin was significantly higher than that before the use (p<0.05, t=19.887). After fasting for 12 h, the blood glucose concentration was more than 11.1 mol/L after 120 min of 50% glucose gavage. The rats lost weight by >50 g, as shown in Figure 1.

Wound Healing in Rats

We performed the statistical results on the wound healing of rats on the 2nd, 11th, and 20th days after wound modeling. The healing rate of rats in the control group was significantly lower than that in the propranolol group (p<0.05). Through the comparison of the two groups, the wound healing rates of the propranolol group and the control group significantly increased with time (p<0.05) (Table II).

Expression of p-ERK1/2 Protein in Rat Wound Tissue

By detecting the expression of p-ERK1/2 protein in the wound tissue of rats on the 2nd, 11th, and 20th days of wound modeling, we found that the expression of the ERK1/2 protein in the wound tissue of the propranolol group was significantly higher than that of the control group on the 11th day (p<0.05), but no significant difference on other time points was detected (p>0.05). The comparison within groups showed that the expression of the p-ERK1/2 protein in the control group gradually increased with time and the expression of the p-ERK1/2 protein in the propranolol group peaked on the 11th day after modeling. In addition, we showed that there was no difference in the p-ERK1/2 protein expression between the two groups on the 2nd day and the 11th day (p>0.05), but there were differences at other time points (p<0.05) (Table III).

Relative Expression of VEGG in Rat Wound Tissue

By detecting the expression of VEGF in the two groups of rats at each time point, we found that the relative expression of VEGF in the pro-

Table II. Wound healing in rats.

Group	2 d	11 d	20 d	F	p
Propranolol (n=21)	15.25±5.24	65.88±11.05*	95.32±4.51*,#	202.735	<0.001
Control (n=21)	4.34±2.19	41.94±8.54*	73.71±9.88*,#	144.423	<0.001
t	5.083	4.535	5.264		
p	0.001	0.001	0.001		

Note: *indicates that there was a difference compared with the 2nd day of the same group (p<0.05), and #indicates that there was a difference compared with the 11th day of the same group (p<0.05).

Table III. Expression of p-ERK1 / 2 protein in rat wound tissue.

Group	2 d	11 d	20 d	F	p
Propranolol (n=21)	0.425±0.098	0.895±0.130*	0.674±0.188*,#	18.773	<0.001
Control (n=21)	0.368±0.084	0.425±0.105	0.575±0.122*,#	7.283	0.005
t	1.168	7.441	1.169		
p	0.265	<0.001	0.265		

Note: *indicates that there was a difference compared with the 2nd day of the same group ($p<0.05$), and #indicates that there was a difference compared with the 11th day of the same group ($p<0.05$).

pranolol group was significantly higher than that in the control group on the 2nd day ($p<0.05$), and the relative expression of VEGF in the propranolol group was significantly higher than that in the control group 11 days after modeling ($p<0.05$). On the 20th day after modeling, the relative expression of VEGF in the propranolol group was not significantly different from that in the control group ($p>0.05$). By comparing the two groups, it was found that there was a difference between the 2nd day, the 20th day, and the 11th day in the wound tissue of propranolol rats ($p<0.05$), and there was no significant difference at other time points ($p>0.05$). The results of the control group showed that there was a difference between the 11th, 20th, and the 2nd day ($p<0.05$), and no significant difference was detected at other time points ($p>0.05$) (Table IV).

Expression of Serum IL-6 and TNF-α in Rats

The expressions of IL-6 and TNF-α in the wound tissue of the two groups were detected by ELISA. There was no significant difference in the expressions of IL-6 and TNF-α on the 2nd day ($p>0.05$). On the 11th and 20th days, the expressions of IL-6 and TNF-α in the propranolol group were significantly higher than those in the control group. On the 20th day, the expressions of IL-6 and TNF-α in the propranolol group were significantly higher than those in the control group, and the difference was statistically significant ($p<0.05$). The intra-group comparison found that the expressions of IL-6 and TNF-α in the propranolol group reached the peak on the 11th day and then gradually decreased. (and) The IL-6 also significantly decreased on the 2nd and 20th days compared with the 11th day ($p<0.05$). There was no significant difference between other time points ($p>0.05$), while the expression of TNF-α in the group showed a significant difference at each time point ($p<0.05$). In the control group, IL-6 and TNF-α reached the peak on the 11th day, and then gradually decreased. The expression of IL-6 was different between the 11th and the 2nd days ($p<0.05$), and there was no significant difference at other time points ($p>0.05$). The TNF-α expression was different at each time point ($p<0.05$) (Table V, VI).

Discussion

Some studies[11,12] have shown that skin wound healing is affected by a variety of factors, including infection, drugs, vascular neuropathy, and immune dysfunction. Clinically, the wounds are often divided into acute wounds and chronic wounds. The acute wounds often refer to the process from the damage to the smooth completion of healing, while the chronic wounds are wounds that are unable to reach the anatomical and functional integrity through normal, orderly, and timely repair processes under various factors,

Table IV. Relative expression of VEGF in rat wound tissue.

Group	2 d	11 d	20 d	F	p
Propranolol (n=21)	1.364±0.189	1.617±0.235*	1.192±0.162#	8.190	0.003
Control (n=21)	0.945±0.144	1.184±0.158*	1.234±0.172*	6.655	0.007
t	4.666	4.046	0.470		
p	0.001	0.002	0.647		

Note: *indicates that there was a difference compared with the 2nd day of the same group ($p<0.05$), and #indicates that there was a difference compared with the 11th day of the same group ($p<0.05$).

Table V. Relative expression of VEGF in rat wound tissue.

Group	2 d	11 d	20 d	F	p
Propranolol (n=21)	167.15±34.58	355.91±113.41*	194.86±41.40#	18.947	<0.001
Control (n=21)	184.52±37.22	245.58±55.54*	143.54±30.84#	10.214	0.001
t	0.905	2.312	2.630		
p	0.384	0.040	0.022		

Note: *indicates that there was a difference compared with the 2nd day of the same group ($p<0.05$), and #indicates that there was a difference compared with the 11th day of the same group ($p<0.05$).

Table VI. Comparison of relative expression of TNF-αin wound tissue of two groups of rats [pg/mL].

Group	2 d	11 d	20 d	F	p
Propranolol (n=21)	149.84±30.71	319.47±67.58*	155.72±35.84*,#	17.443	<0.001
Control (n=21)	143.44±27.58	241.95±37.74*	98.75±26.42*,#	45.159	<0.001
t	0.410	2.650	3.385		
p	0.689	0.021	0.005		

Note: *indicates that there was a difference compared with the 2nd day of the same group ($p<0.05$), and #indicates that there was a difference compared with the 11th day of the same group ($p<0.05$).

and are clinically more common in acne, vascular disease, and metabolic diseases. Among them, diabetic ulcer is the most common[13].

Diabetes mellitus is one of the most common metabolic diseases in the world. As the living standards have been improved in recent years, the incidence of diabetes is increasing year by year[14,15]. Currently, the mechanism of diabetic refractory wounds is still unclear. A conventional theory shows that ischemia, infection, and neuropathy are the three main causes of diabetic refractory wounds[16]. Romana-Souz[17] showed that, by adding propranolol to drinking water in diabetic rats induced by streptozotocin, the cell proliferation and vascular density were significantly higher in the wound area of rats, compared with those in the control group using normal water. The propranolol could accelerate the healing rate of wounds in diabetic rats. However, it is unclear how propranolol promotes wound healing in diabetic rats. Therefore, in this study, we explored the specific mechanism of propranolol on wound healing in diabetic rats and provided some ideas for clinical treatment.

Propranolol, also known as Inderal, is commonly used for the treatment of cardiovascular diseases such as arrhythmia. It is a non-selective β-adrenergic blocker according to its pharmacological effects[18]. The β-adrenergic receptor is widely expressed in various organs of the body and is an important vascular and neuroendocrine regulator[19]. A study has shown that propranolol can be divided into oral and injectables, but because of the inconvenient use and the strict control of the dose, the injectables were less used. In addition, there are wide differences in the propranolol tolerance, which requires an individualized bioavailability of the drug, so there are more studies about the topical dosage[20]. Ointment refers to a semi-solid external preparation, with a suitable consistency prepared by uniformly mixing a drug and a suitable substrate[21]. According to a study[22] that compared 1% propranolol ointment with oral drugs in the treatment of children with superficial hemangiomas, the ointment is significantly better than oral medication. In this study, we used propranolol ointment to treat wound healing in diabetic rats. It was found that the healing rate of the control group was significantly lower than that of the propranolol group at each time point, indicating that propranolol can promote wound healing rate in diabetic rats. Ali et al[23] showed that it is effective to promote wound healing in patients with severe burns by adding propranolol (oral). However, it is unclear how it specifically promotes wound healing in diabetic rats. ERK1/2 is a serine/threonine protein kinase that plays an important role in the MAPK/ERK signaling pathway. Sun et al[24] showed that ERK1/2 is present in the cytoplasm in the inactivated state and phosphorylated once activated. If rapidly transferred to the nucleus, a variety of transcription factors can be activated by phosphorylation, thereby promoting cell proliferation,

invasion, differentiation, inhibition of apoptosis, and regulation of angiogenesis. In this study, by detecting the expression of p-ERK1/2 protein in rat wound tissue at each time point, we found that the expression of p-ERK1/2 protein in the control group gradually increased with time, while the expression of p-ERK1/2 protein in the propranolol group was peaked on the 11th day, and it was significantly increased in the propranolol group compared with that of control group on the 11th day. There was no significant difference at other time points. As a highly specific vascular endothelial growth factor, VEGF promotes vascular permeability, extracellular matrix degeneration, vascular endothelial cell migration, proliferation, and angiogenesis[25]. Xu et al[26] indicated that VEGF can stimulate endothelial cell proliferation and differentiation through p-ERK1/2. Therefore, by detecting the relative expression of VEGF mRNA in rat tissues, it was found that the expression of VEGF was significantly increased on the 2nd day in the propranolol group compared with the control group and reached the peak on the 11th day. There was no significant difference in the expression of VEGF between the two groups on the 20th day. The combination of rat healing rate and p-ERK1/2 protein expression indicated that propranolol promoted the expression of the p-ERK1/2 protein on the 2nd day after modeling, which increased the expression of VEGF. On the 11th day after modeling, the expressions of VEGF and p-ERK1/2 protein in the propranolol group were significantly increased, and the wound healing rate in the rats was also increased. However, when modeling for 20 days, the expression of VEGF in the propranolol group was significantly decreased, while the expression of the p-ERK1/2 protein was reduced. Through the observation of the wound healing rate, we detected that the wounds in the propranolol group have basically healed, which indicates that propranolol can promote the synthesis of extracellular signal-regulated kinase through ERK1/2 phosphorylation, thereby regulating the expression of VEGF and accelerating wound healing in rats. We also detected the expressions of IL-6 and TNF-α in the serum of rats. IL-6 and TNF-α are the classic indicators of inflammatory factors, and their increased expression can directly reflect the inflammation of the body[27,28]. The expressions of IL-6 and TNF-α were not significantly different between the two groups on the 2nd day after modeling, while the expressions of IL-6 and TNF-α in the propranolol group were significantly higher than those of the control group on the 11th day. On the 20th day, the expressions of IL-6 and TNF-α were significantly decreased in the propranolol group. This suggests that propranolol has a role in promoting the development of inflammatory reactions. Chen et al[29] showed that propranolol can promote the secretion of TNF-α by macrophages, which is similar to our findings. This indicates that propranolol can improve wound healing in diabetic rats, but it can also induce the occurrence of the inflammatory reaction in rats.

This study found that propranolol can promote wound healing in diabetic rats and increase the expression of VEGF by ERK1/2 protein phosphorylation to accelerate wound healing in rats. However, there are still some limitations to this study. Firstly, we set up fewer time points. It is not clear how the expressions of p-ERK1/2 and VEGF in rat wound tissues are changed during the treatment. Secondly, the groups were not designed with different doses of propranolol, and it is unclear whether different measurements have an effect on wound healing speed. Finally, propranolol can stimulate the occurrence of the inflammatory reaction in rats. Whether it can reduce the occurrence of this reaction by combining other drugs needs to be explored. Therefore, we hope to add more time points and groups with different doses of propranolol in future researches and to reduce the occurrence of the inflammatory reaction by combining other anti-inflammatory drugs.

Conclusions

We showed that propranolol can accelerate the healing of diabetic wounds by regulating the expression of VEGF by phosphorylation of ERK1/2 protein, thereby treating the healing of chronic wounds in diabetes.

Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Eming SA, Martin P, Tomic-Canic M. Wound repair and regeneration: mechanisms, signaling, and translation. Sci Transl Med 2014; 6: 265sr266.

2) Lyu GZ, Yang ML. Lay further emphasis on cause analysis and non-surgical treatment of chronic

refractory wound. Zhonghua Shao Shang Za Zhi 2017; 33: 68-71.

3) American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2013; 36: S67-74.

4) Bainbridge KE, Hoffman HJ, Cowie CC. Diabetes and hearing impairment in the United States: audiometric evidence from the National Health and Nutrition Examination Survey, 1999 to 2004. Ann Intern Med 2008; 149: 1-10.

5) Xu Y, Wang L, He J, Bi Y, Li M, Wang T, Wang L, Jiang Y, Dai M, Lu J, Xu M, Li Y, Hu N, Li J, Mi S, Chen CS, Li G, Mu Y, Zhao J, Kong L, Chen J, Lai S, Wang W, Zhao W, Ning G, 2010 China Noncommunicable Disease Surveillance Group. Prevalence and control of diabetes in Chinese adults. JAMA 2013; 310: 948-959.

6) Kin NW, Sanders VM. It takes nerve to tell T and B cells what to do. J Leukoc Biol 2006; 79: 1093-1104.

7) Mialet-Perez J, Green SA, Miller WE, Liggett SB. A primate-dominant third glycosylation site of the beta2-adrenergic receptor routes receptors to degradation during agonist regulation. J Biol Chem 2004; 279: 38603-38607.

8) Shilpakar R, Lemperle G, Mentzel T, Shakya J, Bhandari SB. Unexpected effect of propranolol and prednisolone on infantile facial rhabdomyosarcoma. J Pediatr Hematol Oncol 2017; 39: e460-e462.

9) Ying Y, Luo J. Salidroside promotes human periodontal ligament cell proliferation and osteocalcin secretion via ERK1/2 and PI3K/Akt signaling pathways. Exp Ther Med 2018; 15: 5041-5045.

10) Xiao XL, Wang ZY, Tang HJ, Liu XD. Effects of fuhuang shengji yuchuang ointment on expressions of types I and III collagens in granulation tissue of wound in rats with diabetes. Zhong Xi Yi Jie He Xue Bao 2009; 7: 366-371.

11) Lee EJ, Huh BK, Kim SN, Lee JY, Park CG, Mikos AG, Choy YB. Application of materials as medical devices with localized drug delivery capabilities for enhanced wound repair. Prog Mater Sci 2017; 89: 392-410.

12) Zeng RX, He JY, Zhang YL, Liu XX, Zhang Y, Tang Q. Experimental study on repairing skin defect by tissue-engineered skin substitute compositely constructed by adipose-derived stem cells and fibrin gel. Eur Rev Med Pharmacol Sci 2017; 21 (3 Suppl): 1-5.

13) Han G, Ceilley R. Chronic wound healing: a review of current management and treatments. Adv Ther 2017; 34: 599-610.

14) Wang L, Gao P, Zhang M, Huang Z, Zhang D, Deng Q, Li Y, Zhao Z, Qin X, Jin D, Zhou M, Tang X, Hu Y, Wang L. Prevalence and ethnic pattern of diabetes and prediabetes in China in 2013. JAMA 2017; 317: 2515-2523.

15) Schaper NC, Andros G, Apelqvist J, Bakker K, Lammer J, Lepantalo M, Mills JL, Reekers J, Shearman CP, Zierler RE, Hinchliffe RJ, International Working Group on Diabetic foot. Specific guidelines for the diagnosis and treatment of peripheral arterial disease in a patient with diabetes and ulceration of the foot 2011. Diabetes Metab Res Rev 2012; 28 Suppl 1: 236-237.

16) Alavi A, Sibbald RG, Mayer D, Goodman L, Botros M, Armstrong DG, Woo K, Boeni T, Ayello EA, Kirsner RS. Diabetic foot ulcers: Part I. Pathophysiology and prevention. J Am Acad Dermatol 2014; 70: 1-18; quiz 19-20.

17) Romana-Souza B, Santos JS, Monte-Alto-Costa A. Beta-1 and beta-2, but not alpha-1 and alpha-2, adrenoceptor blockade delays rat cutaneous wound healing. Wound Repair Regen 2009; 17: 230-239.

18) Zhou W, He S, Yang Y, Jian D, Chen X, Ding J. Formulation, characterization and clinical evaluation of propranolol hydrochloride gel for transdermal treatment of superficial infantile hemangioma. Drug Dev Ind Pharm 2015; 41: 1109-1119.

19) Kim MH, Gorouhi F, Ramirez S, Granick JL, Byrne BA, Soulika AM, Simon SI, Rivkah Isseroff R. Catecholamine stress alters neutrophil trafficking and impairs wound healing by β2-adrenergic receptor-mediated upregulation of IL-6. J Invest Dermatol 2014; 134: 809-817.

20) Léauté-Labrèze C, Hoeger P, Mazereeuw-Hautier J, Guibaud L, Baselga E, Posiunas G, Phillips RJ, Caceres H, Lopez Gutierrez JC, Ballona R, Friedlander SF, Powell J, Perek D, Metz B, Barbarot S, Maruani A, Szalai ZZ, Krol A, Boccara O, Foelster-Holst R, Febrer Bosch MI, Su J, Buckova H, Torrelo A, Cambazard F, Grantzow R, Wargon O, Wyrzykowski D, Roessler J, Bernabeu-Wittel J, Valencia AM, Przewratil P, Glick S, Pope E, Birchall N, Benjamin L, Mancini AJ, Vabres P, Souteyrand P, Frieden IJ, Berul CI, Mehta CR, Prey S, Boralevi F, Morgan CC, Heritier S, Delarue A, Voisard JJ. A randomized, controlled trial of oral propranolol in infantile hemangioma. N Engl J Med 2015; 372: 735-746.

21) Neri I, Baraldi C, Balestri R, Piraccini BM, Patrizi A. Topical 1% propranolol ointment with occlusion in treatment of pyogenic granulomas: an open-label study in 22 children. Pediatr Dermatol 2018; 35: 117-120.

22) Wahab S M A, Almetaher H A, Fayad H, Elhalaby E. Oral versus topical propranolol for management of superficial infantile hemangiomas: a comparative study. Ann Pediatr Surg 2017; 13: 1-7.

23) Ali A, Herndon DN, Mamachen A, Hasan S, Andersen CR, Grogans RJ, Brewer JL, Lee JO, Heffernan J, Suman OE, Finnerty CC. Propranolol attenuates hemorrhage and accelerates wound healing in severely burned adults. Crit Care 2015; 19: 217.

24) Sun Y, Liu WZ, Liu T, Feng X, Yang N, Zhou HF. Signaling pathway of MAPK/ERK in cell proliferation, differentiation, migration, senescence and apoptosis. J Recept Signal Transduct Res 2015; 35: 600-604.

25) Chamorro-Jorganes A, Lee MY, Araldi E, Landskroner-Eiger S, Fernández-Fuertes M, Sahraei M, Quiles Del Rey M, van Solingen C, Yu J, Fernández-Hernando C, Sessa WC, Suárez Y. VEGF-induced expression of miR-

17-92 cluster in endothelial cells is mediated by ERK/ELK1 activation and regulates angiogenesis. Circ Res 2016; 118: 38-47.

26) Xu J, Liu X, Jiang Y, Chu L, Hao H, Liua Z, Verfaillie C, Zweier J, Gupta K, Liu Z. MAPK/ERK signalling mediates VEGF-induced bone marrow stem cell differentiation into endothelial cell. J Cell Mol Med 2008; 12: 2395-2406.

27) De Simone V, Franze E, Ronchetti G, Colantoni A, Fantini MC, Di Fusco D, Sica GS, Sileri P, MacDonald TT, Pallone F, Monteleone G, Stolfi C. Th17-type cytokines, IL-6 and TNF-α synergistically activate STAT3 and NF-kB to promote colorectal cancer cell growth. Oncogene 2015; 34: 3493-3503.

28) Charrad R, Berraies A, Hamdi B, Ammar J, Hamzaoui K, Hamzaoui A. Anti-inflammatory activity of IL-37 in asthmatic children: correlation with inflammatory cytokines TNF-α, IL-beta, IL-6 and IL-17A. Immunobiology 2016; 221: 182-187.

29) Seyedi S, Andalib A, Rezaei A, Hosseini SM, Mohebbi SR, Zali MR, Vafai M, Behboo R, Tabatabaei SA, Shahabi S. The effects of isoproterenol and propranolol on cytokine profile secretion by cultured tumor-infiltrating lymphocytes derived from colorectal cancer patients. Cell J 2012; 13: 281-289.