

Original Research Article

Effect of allogeneic platelet-rich plasma on the healing of ulcer wound surface in streptozotocin-induced diabetic rats

Yueze Chen¹, Xiaowei Liu², Kesu Hu^{3*}

¹College of Medicine of Nantong University, Nantong, ²Department of Orthopedics, Xishan People's Hospital of Wuxi, Wuxi,

³Department of Burn and Plastic Surgery, Affiliated Hospital of Nantong University, Nantong, China

*For correspondence: **Email:** gc-sh@163.com; **Tel:** +86-013806290911

Sent for review: 5 November 2022

Revised accepted: 26 August 2023

Abstract

Purpose: To evaluate the effect of allogeneic platelet-rich plasma (PRP) on ulcer wound surface in streptozotocin-induced diabetic rats.

Methods: A total of 20 male Sprague-Dawley (SD) rats were procured as skin ulcer as well as diabetes models, and randomly divided into control and PRP groups, respectively. Their wound surfaces were smeared with 0.9 % normal saline or an equal concentration of allogeneic PRP, respectively, and the pathological changes in the wound tissues were examined with the aid of hematoxylin-eosin (H&E) staining. Also, the expression levels of transforming growth factor- β 1 (TGF- β 1), matrix metalloproteinase-2 (MMP-2), and tissue inhibitor of metalloproteinase-2 (TIMP-2) in wound tissues were determined using enzyme-linked immunosorbent assay (ELISA).

Results: Both purulent exudation and swelling of the wound surface were milder in the PRP group than in the control group, and the PRP group showed greater redness on the wound surface and more pronounced signs of epithelialization of the wound surface and its margins ($p < 0.05$). Wound healing rate in PRP group was higher than in the control group while the number of fibroblasts and new microvessels in the wound surface in the PRP group were greater than in the control group, accompanied by slighter inflammatory response compared to the control group. Furthermore, the PRP group expressed lower MMP-2, MMP-2/TIMP-2 ratio, and higher TGF- β 1 and TIMP-2 levels than in the control group ($p < 0.05$).

Conclusion: Allogeneic PRP treatment contributes to the healing of ulcer wound surface in diabetic rats, a process mediated by TGF- β 1/MMP-2/TIMP-2 signaling pathway. Thus, PRP is a potential therapeutic agent for the management of diabetic ulcer wounds.

Keywords: Platelet-rich plasma (PRP), Diabetic ulcer wound, Wound surface healing, Transforming growth factor- β 1 (TGF- β 1)

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Diabetes mellitus is a clinically common chronic metabolic disease with an increasing incidence.

According to relevant data, approximately 630 million people worldwide are expected to suffer from diabetes mellitus by 2045 [1]. When there is a failure to control the blood glucose of diabetic

patients due to a variety of factors, the disease keeps progressing and a series of acute and chronic complications occur. Diabetic ulcer, especially diabetic foot ulcer, is one of the most important chronic complications, and it often results in amputation and even death in critical cases. Thus, it affects patients' quality of life and is a heavy economic burden to the patient's family [2].

Platelet-rich plasma (PRP) refers to the plasma and its concentrate at a concentration 3-7 times higher than the baseline platelet concentration obtained following repeated separation and concentration of fresh peripheral whole blood. PRP has been widely applied in numerous fields like orthopedics, burns and plastic surgery, and cosmetic dermatology, with significant clinical efficacy [3]. In recent studies, PRP has been applied in the treatment of diabetic ulcers [4]. Previous research have stated the unique merits of PRP in the treatment of diabetic ulcers, such as reduction in the length of hospital stay, lower hospitalization expenses, and a decrease in the expression of some inflammatory indicators [5]. However, the specific mechanism of action of PRP in the treatment of diabetic ulcers remains unclear. In this study, the effect of PRP on diabetic ulcer wound surface in established diabetic ulcer rat models was investigated. Furthermore, the expression levels of transforming growth factor- β 1 (TGF- β 1), matrix metalloproteinase-2 (MMP-2) and tissue inhibitor of metalloproteinase-2 (TIMP-2) in granulation tissues of the wound surface were measured at different intervention time points.

EXPERIMENTAL

Laboratory animals

Twenty Sprague-Dawley rats (male, 6 week old, 160-220 g) were purchased from Nantong University Animal Center, and fed in a standard environment at 24 ± 2 °C and relative humidity of 50 ± 10 %. The feeding room was disinfected regularly and well-ventilated, and all rats were adaptively fed with common feed in separate cages for 1 week before the experiment, and they had free access to food and water. This study was approved by the Animal Ethics Committee of Nantong University Animal Center (NU-A-01), and followed international guidelines for the study of animals.

Main reagents and instruments

The reagents and instruments used were as follows: streptozotocin (STZ) and bovine thrombin (Sigma, St. Louis, MO, USA); citric

acid, sodium citrate, glucose, calcium chloride, and 0.9 % normal saline (Sichuan Kelun Pharmaceutical Co., Ltd., Chengdu, China); rat TGF- β 1, MMP-2 and TIMP-2 enzyme-linked immunosorbent assay (ELISA) kits (R&D, Minneapolis, MN, USA); a cryogenic high-speed centrifuge (Eppendorf, Hamburg, Germany); pipettes (Thermo Fisher, Waltham, MA, USA); a glucometer and test papers (Roche Diagnostics, Basel, Switzerland); an ordinary optical microscope (Leica, Wetzlar, Germany); an electric shaver (Shanghai Flyco Electric Appliance Co., Ltd., Shanghai, China); an electronic balance (Changzhou Wantai Electrical Appliance Co. Ltd, Changzhou, China); and high-glucose high-fat diet (67.5 % of basal feed, 20% of white sugar, 10 % of lard and 2.5 % of egg yolk) (Shanghai Ruisai Bio-Technology Co. Ltd, Shanghai, China). Experimental tools such as surgical forceps, surgical scissors and scalpels were obtained from SteriLance Medical (Suzhou, China).

Establishment of diabetic rat models

All rats were fed with a high-glucose high-fat diet lasting four weeks, and then starved for 12 h with water available. Later, body weight was measured after starvation and the dose of STZ required to induce diabetes was calculated (30 mg/kg). 1 % STZ solution was prepared following the instruction steps, and injected intraperitoneally one at a time. After 3 days, caudal vein blood was collected and the random blood glucose was measured. Random blood glucose ≥ 16.7 mmol/L indicated that the diabetic rat model was established successfully, otherwise the rats were starved for another 12 h and then injected with STZ solution (10 mg/kg) once more following the measure of the random blood glucose. If random blood glucose still did not meet the standard of ≥ 16.7 mmol/L, the rat was sacrificed [6].

Establishment of diabetic ulcer models

Surgical instruments were prepared and disinfected in advance before experiment. The rats were weighed and anesthetized by intraperitoneal injections of sodium pentobarbital (150 mg/kg), and following a successful anesthetic, depilatory creams were given and an electric shaver was used to shave the surgical area on the back. After the hair was removed completely, the remaining depilatory cream was wiped off with 75 % ethanol. Then the rats were marked using a marking pen on the back along the side (2 cm) of a square cardboard prepared in advance. The rats were then fixed on the operating table with ropes and disinfected

routinely before operation. The skin and subcutaneous tissues along were then incised with the marking line using sterile surgical instruments to create a skin wound surface deep into the fascia layer, with the deep fascia and tissues below it preserved. The wound surface was slightly trimmed in the periphery using fine tissue scissors. The created defective full skin was exposed, and 1 mL of *Staphylococcus aureus* suspension (6×10^{12} /L) was applied on the wound. After they were photographed, the wound surface was covered with sterile gauze, and the four edges were fixed with surgical sutures. A "concave" shape was made with surgical scissors on the gauze to monitor the healing status of the wound surface [7,8].

Preparation of allogeneic PRP

Another 12 healthy SD rats were anesthetized by intraperitoneal injections of sodium pentobarbital (150 mg/kg). Under direct vision, 10 mL of whole blood was drawn from the abdominal aorta by a puncture with a pre-heparinized 5 mL syringe and a disposable venous blood collection needle. Then, measure the platelet count (about 0.46×10^9 / mL) of 1 mL whole blood was measured using a whole blood cell analyzer. The remaining 9 mL whole blood was placed into an anticoagulated centrifuge tube and centrifuged at $200 \times g$, 4°C for 15 min, and formed three layers. The supernatant and interface layer of the mixture were transferred to a new centrifuge tube and centrifuged again ($500 \times g$, 4°C , 10 min). After that, discard the upper platelet-poor plasma (PPP) was discarded, and about 1.85×10^{12} /mL of platelet-rich plasma (PRP) was obtained. When used for tissue repair or wound treatment, PRP is usually mixed with thrombin-calcium agents at a ratio of 10:1 in order to activate the platelets and fibrinogen. After natural coagulation, the mixture turned into platelet-rich gel (PRG), and was used to seal and protect the wound surface, so as to stop bleeding and prevent infection [9,10].

Grouping and procedures

After the above modeling experiment with 20 SD rats, 18 diabetic ulcer rats were finally obtained and randomly divided into control group ($n = 9$) and a PRP group ($n = 9$). The wound surface was smeared with 1 mL of 0.9 % normal saline in the control group, while it was evenly smeared with 1 mL of allogeneic PRP in the PRP group. Then the wound surface was covered with sterile gauze and bandaged in both groups. Thereafter, all rats were returned to individual cages and numbered. Wound dressing was changed every other day, and the wound surface treated in the

same way, *i.e.*, the wound surface was disinfected with bound iodine first, then wiped dry with sterile gauze and routinely bandaged.

Sample collection

At 3, 7 and 14 days after intervention, the rats were weighed and anesthetized using intraperitoneal injections of sodium pentobarbital (150 mg/kg). Following a successful application of anesthetic, the skin tissues of the wound surface and 5 mm around the wound surface was collected. Part of the tissues were randomly taken, washed clean on the surface with PBS, and fixed with 10 % neutral formalin, followed by hematoxylin-eosin (H&E) staining. Healing status was examined under an optical microscope. The remaining tissues were also washed with PBS to remove the surface stains, placed into a 2 mL EP tube and cryopreserved at -80°C . Subsequently, the protein levels of TGF- β 1, MMP-2 and TIMP-2 in wound tissues were determined by ELISA (the ulcer tissues were collected in the same way for ELISA at 1 day after modeling).

Evaluation of parameters/indices

Wound surface healing status

The intent ulcer wound surface was examined with naked eyes and evaluated from many aspects, including the inflammatory response, exudate volume, degree of swelling, color and new granulation tissue formation of the wound surface. A general view on the wound surface was photographed, the current wound area was automatically calculated using ImageJ, and the wound healing rate (WHR) was calculated as the evaluation index for the wound surface healing status, as shown in Equation 1.

$$\text{WHR (\%)} = [(A_i - A_c) / A_i] \times 100 \% \dots \dots (1)$$

where A_i is the initial wound area, and A_c is the current wound area.

Histopathological examination of wound surface

A slice of sample was taken for H&E staining from the wound surface while peri-wound tissues were collected at 3, 7 and 14 days after intervention, and the pathological conditions of the wound surface healing status were photographed.

Biochemical studies

TGF- β 1, MMP-2 and TIMP-2 levels in the wound tissues were determined by ELISA. The

remaining wound tissues (stored at $-80\text{ }^{\circ}\text{C}$) harvested at 1, 3, 7 and 14 days after intervention were taken out, melted at $2\text{--}8\text{ }^{\circ}\text{C}$, and homogenized in PBS (pH 7.4). Then, the mixture was centrifuged. Finally, the concentrations of TGF- β 1, MMP-2 and TIMP-2 in the supernatant were measured by ELISA kits. The operating procedures and indicators were in accordance with ELISA kit manufacturers' instructions.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 22.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. Data were displayed as mean \pm standard deviation (SD). Independent-sample t-test was used to compare the data at the same time-point between the two groups. $P < 0.05$ was considered statistically significant.

RESULTS

Modeling

Twenty male SD rats were initially prepared for modeling. Through 4 weeks of high-glucose high-fat feeding combined with intraperitoneal injection of STZ, the diabetic model was established. Following STZ injection, the blood glucose level significantly rose, but 2 cases of modeled rats failed to reach the required standard (blood glucose $\geq 16.7\text{ mmol/L}$), and 18 diabetic model rats were finally obtained. Then the ulcer wound surface was created on the back of diabetic rats. During this process, the rats all survived in good condition. As a result, the total modeling success rate reached 90%. The mean blood glucose level of rats fed with a high-glucose diet ($25.54 \pm 1.63\text{ mmol/L}$) was far higher than that of normal rats [$5.06 \pm 0.78\text{ mmol/L}$], ($p < 0.05$). About 3 days after surgery, the wound surface turned black, and the skin temperature rose. Redness and swelling occurred in the wound surface and peri-wound area, and necrotic tissues; purulent secretions or necrotic dark crust were observed on the wound surface, and there was no bleeding on the wound surface when pricked by a pin across a length of 5 mm. Overall, the wound surface was consistent with the general ulcer condition based on naked eye observation.

Wound surface dried out and formed a scab

Three days after intervention, purulent exudation of the ulcer wound surface could be seen in both groups, but it was more significant with dry scabs and pus accumulation under the scab in the

control group. At 7 days after intervention, new granulation tissues could be found and the wound surface was bright red in the PRP group, while dry scabs and pigmentation at the wound margin could be seen, and the wound surface appeared lighter in color in control group. After 14 days of intervention, the wound surface became smaller and started to heal, and the wound was filled with granulation tissues in PRP group. In control group, there were dry scabs and pigmentation on the wound surface (Figure 1).



Figure 1: Comparison of wound surface healing status between the two groups at different time points

Wound healing rate

At 3 days after intervention, the wound healing rate in the PRP group was higher than that in the control group, but the difference was not significant ($p > 0.05$). At 7 and 14 days after intervention, the wound healing rate in PRP group was significantly higher than in control group ($p < 0.01$), suggesting that PRP exhibited excellent healing effect (Table 1).

Table 1: Comparison of wound healing rate at different time points between the two groups (%)

Group	Day 3 after intervention	Day 7 after intervention	Day 14 after intervention
Control	1.46 ± 0.51	12.92 ± 1.29	31.46 ± 2.00
PRP	2.33 ± 0.83	$25.83 \pm 2.19^*$	$78.54 \pm 0.94^*$

Note: * $P < 0.05$ vs. control group

Morphological features of wound surface

At 3 days after intervention, erythrocyte exudation (yellow) was observed under the microscope in both groups, but the manuscript was milder in the PRP group. The inflammatory response dominated by neutrophils (blue) occurred in both groups, and there were new microvessels in the PRP group. At 7 days after intervention, the inflammatory response was alleviated, and a large number of fibroblasts

(black) and new microvessels could be seen in the PRP group. In the control group, erythrocyte exudation, severe inflammatory response and a small number of fibroblasts could be seen. At 14 days after intervention, granulation tissues (green) were found in both groups, and they were denser in the PRP group (Figure 2).

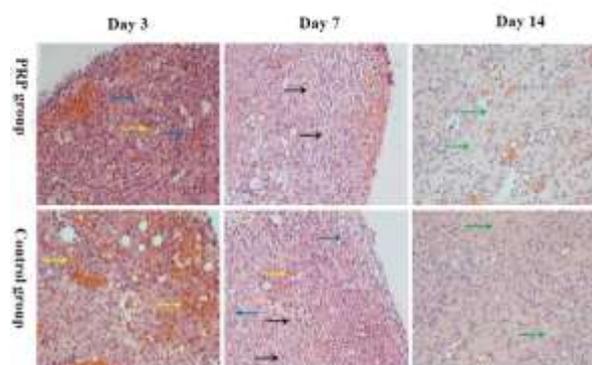


Figure 2: Comparison of pathological conditions of wound surface between the two groups at different time-points

TGF- β 1, MMP-2 and TIMP-2 proteins in wound tissues

The TGF- β 1 protein level in wound tissues was significantly higher in the PRP group than in the control group within a certain period (1 and 3 days after intervention; $p < 0.05$). The TGF- β 1 protein level in the wound tissues gradually rose and then decreased in both groups with time, and it was significantly higher in the control group

than that in the PRP group after intervention for 7 days ($p < 0.05$; Table 2). The MMP-2 protein level in the wound tissues was significantly lower in the PRP group than in the control group, and the difference was statistically significant after intervention for 3 days ($p < 0.05$). The MMP-2 protein level in the wound tissues gradually rose and then declined in both groups with time (Table 3). The TIMP- protein expression level of wound tissues in the PRP group was significantly higher than in control group on day 3 of the intervention ($p < 0.05$). The TIMP-2 protein level in wound tissues gradually rose in both groups with time (Table 4). The MMP-2/TIMP-2 ratio was calculated based on the ELISA results of MMP-2 and TIMP-2. The MMP-2/TIMP-2 ratio in wound tissues in the PRP group was lower than in the control group at different time points ($p < 0.05$). Specifically, the MMP-2/TIMP-2 ratio of wound tissues gradually decreased over time in the PRP group, while in the control group, it fluctuated at a high level (compared to the PRP group) for a period of time and began to decline after intervention for 7 days (Table 5). After PRP treatment, TGF- β 1 had a strong positive correlation (98.97 %) with MMP-2, and the correlation coefficient was 0.9897; it also had a strong positive correlation (86.37 %) with TIMP-2, and the correlation coefficient was 0.8637. There was a weak negative correlation (32.67 %) between TGF- β 1 and MMP-2/TIMP-2, and the correlation coefficient was -0.3267. No correlation was found between MMP-2 and TIMP-2, and the (correlation coefficient was -0.1889).

Table 2: Levels of TGF- β 1 in wound tissues at different time-points in the two groups (ng/L, mean \pm SD)

Group	Day 1 after intervention	Day 3 after intervention	Day 7 after intervention	Day 14 after intervention
Control	53.22 \pm 5.64	101.34 \pm 6.21	128.56 \pm 5.86**	90.86 \pm 6.34**
PRP	63.35 \pm 6.91*	132.36 \pm 6.26*	73.56 \pm 6.33	30.94 \pm 5.81

* $P < 0.05$ vs. control group, ** $p < 0.05$ vs. PRP group

Table 3: Levels of MMP-2 in wound tissues at different time-points in the two groups (ng/L)

Group	Day 1 after intervention	Day 3 after intervention	Day 7 after intervention	Day 14 after intervention
Control	22.35 \pm 3.56	47.63 \pm 2.67	69.74 \pm 2.83	30.68 \pm 2.87
PRP	19.82 \pm 2.79	40.16 \pm 3.54*	57.68 \pm 2.97*	24.79 \pm 2.32*

Note: * $P < 0.05$ vs. control group

Table 4: Levels of TIMP-2 in wound tissues at different time-points in the two groups (ng/L)

Group	Day 1 after intervention	Day 3 after intervention	Day 7 after intervention	Day 14 after intervention
Control	15.36 \pm 2.73	36.96 \pm 3.56	52.37 \pm 4.39	65.93 \pm 5.36
PRP	20.86 \pm 3.96	47.54 \pm 3.52*	70.81 \pm 2.37*	81.57 \pm 6.39*

Note: * $P < 0.05$ vs. control group

Table 5: MMP-2/TIMP-2 ratios in wound tissues at different time-points in the two groups

Group	Day 1 after intervention	Day 3 after intervention	Day 7 after intervention	Day 14 after intervention
Control	1.47 ± 0.12	1.18 ± 0.59	1.32 ± 0.24	0.48 ± 0.09
PRP	0.96 ± 0.07*	0.85 ± 0.08*	0.81 ± 0.06*	0.27 ± 0.03*

* $P < 0.05$ vs. control group

DISCUSSION

Wound healing has always been a topic of concern in the clinical surgery field. Chronic wounds such as bedsores and diabetic foot ulcers are intractable problems in wound healing. The wound surface healing process normally involves four stages namely, hemostasis, inflammation, proliferation and remodeling. A moderate inflammatory response helps resist infections, which is the basis of wound repair [11,12]. However, excessive inflammation is present on the diabetic ulcer wound surface, and the wound surface is in a chronic inflammatory state, with persistent inflammation, a large number of inflammatory factors and mediators, and increased inflammatory cell infiltration [13,14]. Inflammatory cells induce the generation and release of matrix metalloproteinases (MMPs) in the local wound surface, and MMPs further degrade beneficial growth factors in the wound surface, ultimately resulting in difficult healing of the diabetic ulcer wound surface [15,16].

Matrix metalloproteinases (MMPs), a class of zinc-dependent proteolytic enzymes, regulate the complex and orderly physiological repair process by regulating the normal and controllable metabolism of extracellular matrix (ECM), thereby playing an important role in wound healing [17]. MMPs can be classified into 6 types according to their acting substrates, among which collagenase and gelatinase are the most important. MMP-2 as a member of gelatinase, degrades various components, such as collagen type IV and type V, elastin and gelatin (denatured collagen) [18]. MMP-2 regulates ECM, participates in vascular remodeling and tissue reconstruction, and promotes the growth of granulation tissues. Tissue inhibitors of metal protease (TIMPs), which are specific inhibitors of MMPs, inhibit the activation of MMPs directly by preventing zymogen activation [19]. TIMP-2 is an endogenous inhibitor of MMP-2, which exerts a direct inhibitory effect on MMP-2. With the expression of MMP-2 in tissues, TIMP-2 is also expressed so as to inhibit the expression and activity of MMP-2 and prevent the excessive destruction caused by MMP-2, which is rarely affected by other cytokines. In addition, TIMP-2 also participates in the regulation of cell proliferation and angiogenesis directly [18]. It has

been proven in several studies that TIMP-2 restricts ECM proteolysis. Overexpression of TIMP-2 breaks the balanced MMP-2/TIMP-2 that leads to poor wound healing behavior, and causes diseases such as Dupuytren's contracture, a kind of abnormal accumulation of ECM [20]. Studies have shown that there is a higher expression of MMP-2 and a lower expression of TIMP-2 in diabetic ulcers models in normal wound healing, and along with the increase in bacterial species and ulcer grades, the MMP-2 level rises and the MMP-2/TIMP-2 ratio significantly increases, suggesting that MMP-2/TIMP-2 imbalance in diabetic ulcer is one of the possible causes of wound healing disorders [21].

During ulcer wound repair, a variety of cytokines play important roles, among which TGF- β is a representative positive regulatory cytokine [22]. Papanas *et al.* [23] showed that cytokines such as TGF- β contribute to diabetic ulcer healing. There are three members in TGF- β (TGF- β 1, TGF- β 2 and TGF- β 3), among which TGF- β 1 possesses the ability to promote matrix synthesis and fibroblast growth plays an essential role in ulcer wound repair. As a novel therapeutic tool applied to wound treatment in recent years, the activation of PRP recruits a variety of cytokines like TGF- β 1. TGF- β 1 has been shown to be able to promote collagen formation [24], chemotaxis of macrophages, and fibroblasts to wounds [25]. Moreover, TGF- β 1 facilitates ulcer tissue healing in a multidimensional way through its action on inflammatory response, angiogenesis and collagen accumulation [26]. Existing studies suggest that TGF- β 1 may be implicated in the matrix synthesis and degradation by regulating the relative expression levels of MMP-2/TIMP-2, exerting a positive regulatory effect [27]. However, basic studies are still lacking on this aspect.

In this study, the results showed that both indicators of the ulcer wound healing status, *viz*, wound healing rate and wound healing speed were significantly higher in PRP group than in control group. Compared to control group, PRP group displayed an increased number of structural cells in wound tissues, manifested as an obvious fibroblast division with more fibrous tissues and new blood vessels, and fewer inflammatory cells. Within the 14-day observation

period of this study, when compared to control group, PRP group expressed a lower MMP-2 level, but higher levels of TGF- β 1 and TIMP-2, and lower MMP-2/TIMP-2 ratio ($p < 0.05$). Furthermore, in PRP group, TGF- β 1 was positively correlated with TIMP-2 but negatively correlated with MMP-2/TIMP-2 ratio.

CONCLUSION

This study proves that PRP contributes to the healing of rat diabetic ulcer wound surface, increases the TGF- β 1 and TIMP-2 levels in ulcer tissues, and decreases the MMP-2 level, thereby accelerating the recovery of the wound surface. Animal experiments suggest that PRP perhaps increases the activated TGF- β 1, inhibits its downstream MMP-2 and promotes TIMP-2 expression which can control matrix synthesis and degradation, thereby facilitating tissue healing. Therefore, PRP is a potential agent for the management of diabetic wound ulcers in humans.

DECLARATIONS

Acknowledgements

None provided.

Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

Open Access

This is an Open Access article that uses a funding model which does not charge readers or

their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

REFERENCES

1. Vukmirovic D, Krajnovic D, Odalovic M. Perceptions of pharmacists and other healthcare professionals on marketed mobile applications used for self-management by type 2 diabetic patients: A systematic review. *Trop J Pharm Res* 2023; 22(4):917-925 doi: 10.4314/tjpr.v22i4.27
2. Fiskin G, Sahin N. Nonpharmacological Management of Gestational Diabetes Mellitus: Diaphragmatic Breathing Exercise. *Altern Ther Health M* 2021; 27: 90-96.
3. Abdulla AK, Rebai T, Al-Delemi DHJ. Protective Effects of Autologous Platelet-Rich Plasma (PRP) on the Outcome of Cryopreservation in Rabbit Sperm. *Cell Mol Biol* 2022; 68(4): 113-121.
4. Qian Z, Wang H, Bai Y, Wang Y, Tao L, Wei Y, Fan Y, Guo X, Liu H. Improving Chronic Diabetic Wound Healing through an Injectable and Self-Healing Hydrogel with Platelet-Rich Plasma Release. *ACS Appl Mater Interfaces*. 2020 Dec 16;12(50):55659-55674.
5. Hu Z, Qu S, Zhang J, Cao X, Wang P, Huang S, Shi F, Dong Y, Wu J, Tang B, Zhu J. Efficacy and Safety of Platelet-Rich Plasma for Patients with Diabetic Ulcers: A Systematic Review and Meta-analysis. *Adv Wound Care (New Rochelle)*. 2019 Jul 1;8(7):298-308.
6. Chatzigeorgiou A, Halapas A, Kalafatakis K, Kamper E. The use of animal models in the study of diabetes mellitus. *In Vivo* 2009; 23(2): 245-258.
7. Gao S, Shen Y, Geng F, Li Y, Gao J. [Research progress on the animal models and treatment strategies of diabetic foot ulcer]. *Zhejiang Da Xue Xue Bao Yi Xue Ban* 2017; 46(1): 97-105.
8. Lee JH, Ja KJ, Shin HB, Jung HW, Lee YK, Yeo ED, Seok YS. Comparative efficacy of silver-containing dressing materials for treating MRSA-Infected wounds in rats with Streptozotocin-induced diabetes. *Wounds* 2013; 25(12): 345-354.
9. Li W, Feng R. [Preparation of platelet-rich plasma gel and its effect on skin flap survival of rat]. *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi* 2012; 26(1): 64-69.
10. Venter NG, Marques RG, Santos JS, Monte-Alto-Costa A. Use of platelet-rich plasma in deep second- and third-degree burns. *Burns* 2016; 42(4): 807-814.
11. Leyva-Gomez G, Gonzalez-Torres M, Alcalá-Alcalá S, Bernal-Chavez SA, Morales-Morfin JC, Gonzalez-Del Carmen M, Sharifi-Rad J, Figueroa-Gonzalez G, Reyes-Hernandez OD, Del Prado-Audelo ML, et al. Development of films from natural sources for infections during wound healing. *Cell Mol Biol* 2021; 67(1): 96-100.

12. Hunt TK, Hopf H, Hussain Z. Physiology of wound healing. *Adv Skin Wound Care* 2000; 13(2 Suppl): 6-11.
13. Pradhan Nabzdyk L, Kuchibhotla S, Guthrie P, Chun M, Auster ME, Nabzdyk C, Deso S, Andersen N, Gnardellis C. Expression of neuropeptides and cytokines in a rabbit model of diabetic neuroischemic wound healing. *J Vasc Surg* 2013; 58(3): 766-75.e12.
14. Baltzis D, Eleftheriadou I, Veves A. Pathogenesis and treatment of impaired wound healing in diabetes mellitus: new insights. *Adv Ther* 2014; 31(8): 817-836.
15. Salazar JJ, Ennis WJ, Koh TJ. Diabetes medications: Impact on inflammation and wound healing. *J Diabetes Complications* 2016; 30(4): 746-752.
16. Mirza RE, Fang MM, Weinheimer-Haus EM, Ennis WJ, Koh TJ. Sustained inflammasome activity in macrophages impairs wound healing in type 2 diabetic humans and mice. *Diabetes* 2014; 63(3): 1103-1114.
17. Cui N, Hu M, Khalil RA. Biochemical and Biological Attributes of Matrix Metalloproteinases. *Prog Mol Biol Transl Sci.* 2017; 147:1-73.
18. Ou T, Wang W, Yong H, Hao H, Wang R, Dai X, Wang D, Li Y. Liraglutide Plus Dapagliflozin for High Uric Acid and Microalbuminuria in Diabetes Mellitus Complicated With Metabolic Syndrome. *Altern Ther Health M* 2022; 28(6): 14-21.
19. Murphy G. Matrix metalloproteinases and their inhibitors. *Acta Orthop Scand Suppl* 1995; 266: 55-60.
20. Ratajczak-Wielgomas K, Gosk J, Rabczynski J, Augoff K, Podhorska-Okolow M, Gamian A, Rutowski R. Expression of MMP-2, TIMP-2, TGF-beta1, and decorin in Dupuytren's contracture. *Connect Tissue Res* 2012; 53(6): 469-477.
21. Shirinsky IV, Kalinovskaya NY, Filatova K, Shirinsky VS. Pleiotropic Effects of *Comarum palustre* L. in Patients with Osteoarthritis and Diabetes Mellitus with High Comorbidity Burden: An Exploratory Study. *Altern Ther Health M* 2021; 27: 80-84.
22. Alavi M, Zorab MM, Ashengroph M, Aljelehawy QHA, Kahrizi D. Antibacterial and wound healing applications of curcumin in micro and nano-scaffolds based on chitosan, cellulose, and collagen. *Cell Mol Biol* 2022; 68(3): 9-14.
23. Papanas N, Maltezos E. Becaplermin gel in the treatment of diabetic neuropathic foot ulcers. *Clin Interv Aging* 2008; 3(2): 233-240.
24. Zhang W, Chancey AL, Tzeng HP, Zhou Z, Lavine KJ, Gao F, Sivasubramanian N, Barger PM, Mann DL. The development of myocardial fibrosis in transgenic mice with targeted overexpression of tumor necrosis factor requires mast cell-fibroblast interactions. *Circulation* 2011; 124(19): 2106-2116.
25. Chang S, Lamm SH. Human health effects of sodium azide exposure: a literature review and analysis. *Int J Toxicol* 2003; 22(3): 175-186.
26. Klein MB, Yalamanchi N, Pham H, Longaker MT, Chang J. Flexor tendon healing in vitro: effects of TGF-beta on tendon cell collagen production. *J Hand Surg Am* 2002; 27(4): 615-620.
27. Sun Y, Lyu M, Lu Q, Cheung K, Leung V. Current Perspectives on Nucleus Pulposus Fibrosis in Disc Degeneration and Repair. *Int J Mol Sci.* 2022; 23(12): 6612.