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Original Article

Autologous platelet-rich gel for lower-extremity ischemic ulcers in patients with type 2 diabetes

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Abstract: Objective: To analyze the clinical effect of the autologous platelet-rich gel (APG) in topical treatment of ischemic ulcers of lower extremity in patients with type 2 diabetes mellitus (T2DM). Methods: A total of 76 T2DM patients with ischemic ulcers of lower extremity treated in our hospital were enrolled in this study. They were randomly allocated to receive a routine treatment plus standard topical treatment of ulcers (the control group, n=38) or wet topical APG dressings in ulcers plus routine treatment (the treatment group, n=38). The factors of healing time, wound healing rate, overall response rate, the positive rate of bacterial culture of wound secretions and the degree of wound pain were compared between the two groups at one month after treatment. And the levels of transforming growth factor β1 (TGFβ1), insulin-like growth factor (IGF)-1, platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) in granulation tissues of ischemic ulcers were also compared between the two groups. In addition, the factors of scars (the Vancouver scar scale, VSS) and adverse reactions were compared between the two groups. Results: When compared with the control group, shorter healing time, a higher wound healing rate, a rising overall response rate and a lower rate of positive bacterial culture of wound secretions were observed in the experimental group (All P<0.05), and the scores of VAS and VSS were lower in the experimental group than in the control group (P<0.05). The levels of TGFβ1, IGF-1, PDGF and EGF in granulation tissues of ischemic ulcers were higher in the experimental group than in the control group (All P<0.05). However, the occurrence of adverse reactions did not differ significantly between the two groups (P>0.05). Conclusion: Topical APG therapy may be an effective and safe adjuvant therapy in management of lower-extremity ischemic ulcers in patients with T2DM.

Keywords: Autologous platelet-rich gel, type 2 diabetes mellitus, lower-extremity ischemic ulcers, clinical efficiency

Introduction

The prevalence of chronic skin ulcers is increasing on a yearly basis, and has become one of the common clinical complications of diabetes [1]. Diabetic chronic skin ulcers occur primarily in the lower extremities, especially the foot, exerting a great detrimental impact on the quality of life (QOL) of the patients with diabetes [1]. As proven in previous clinical practices, it is difficult to heal diabetic patients with ischemic ulcers of lower extremities, most of which develop into refractory skin ulcers. As a result, diabetic refractory ischemic ulcers of lower extremity have aroused increasing interest of clinicians [2]. Over the recent years, great progress has made in the techniques of wound healing, among which autologous platelet-rich gel (APG) has attracted the most substantial attention. APG has been extensively used in such clinical wound healing as refractory wound, trauma ulcers and pressure ulcers after fracture therapy [3-5]. It has demonstrated that the concentrations of various growth factors in APG are high, which is favorable for tissue repairs and regeneration as well as early healing of ischemic ulcers [4]. Currently, however, the clinical efficacy of APG in the management of lower-extremity ischemic wound/ulcers in diabetic patients is still controversial [6, 7]. Thus, this study was designed to measure and compare the therapeutic effect of APG in the treatment of ischemic ulcers of lower extremity in diabetic patients.

Materials and methods

Study subjects

Overall, 76 adult diabetic patients treated in our hospital from January 2016 to March 2017

were enrolled in our study as subjects. Among them, 36 were male and 40 were female, with an average age of 41.6±7.8 years. This study was reviewed and approved by the Ethics Committee of our hospital. Among the eligible patients, 73 had foot ulcers (Grade III-IV based on the Wagner Ulcer Grade Classification System), and five had leg ulcers. The patients were included if they had lower-extremity ischemic ulcers; good blood supply in the region adjacent to the ischemic ulcers lesions, lower-extremity segmental blood pressure (ankle-brachial index, ABI) ≥ 0.6; signed written informed consents; continued follow-ups after discharge and complete clinical data were available [8]. Patients were excluded if they had significant dysfunctions in heart, brain, lung, kidney and other organs; serious complications associated with diabetes and systemic infection; poor general conditions or allergies to the study drugs; use of immunosuppressive agents; APG allergies.

Randomization

The patients were randomized into the following two groups in terms of a random number table: the control group (receiving routine and topical standard treatment for ulcers); the treatment group (receiving wet topical APG dressings on ulcers plus routine treatment). Each group had 38 patients.

Ulcer treatment

The control group: After admission, the blood glucose and blood pressure of the patients were under strict control, meeting the standard criteria; they received symptomatic treatment including subcutaneous injection of low-molecular-weight heparin calcium at 0.1 ml/10 kg for anticoagulation once every 12 hours for 7 days; intravenous infusion of ceftriaxone 2.0 once per day, with the treatment time depending on the picture of wound healing and bacterial culture, as well as intravenous infusion of compound vitamin solution along with nutritional support. And complete debridement was performed to remove the erosions and necrotic tissues to expose healthy tissues. The normal saline containing metronidazole solution and gentamicin was used to repeatedly rinse the ulcers/wounds, and a drainage catheter was inserted into the site for decompression. The ulcers/wounds were covered with sterile dressing which was replaced every other day until healing.

The treatment group: The same routine and supportive care and debridement measures were also given to the patients in the treatment group. During the APG treatment, the APG gel was posted on the wounds, replaced once every other week. The wounds were covered with sterile dressing. The external dressing was replaced every other day for one week until healing.

The APG gel was prepared within 30 minutes before each dressing replacement. The preparation was made in the following steps: firstly, 1 ml of anticoagulant sodium citrate was added into the centrifuge tube and then thoroughly mixed with 10 ml of intravenous blood, followed by high-speed centrifugation of the mixture at 1500 bpm for 10 min. After that, the plasma and 1 mm of erythrocytes adjacent to the interface of the mixture were pipetted into another centrifuge tube, and then centrifuged at 3600 bpm for 10 min. After the plasma layer containing very small amounts of non-subsided platelets were pipetted from the top layer, the remaining plasma and haemocytes at the top layer were platelet-rich plasma (PRP). PRP and coagulant (1 ml of calcium chloride plus 1000 U of thrombin) were mixed at the ratio of 1 ml: 200 U. In this way, the newly-made mixture was APG gel.

Outcome measures

Ulcer/wound healing outcomes: At one month after treatment, the factors including healing rate, overall response rate, and bacterial culture of wound secretions were recorded and compared between the treatment group and the control group. The criteria for ulcer/wound healing were as follows: healing, the ulcers/wounds where 100% of epithelial healing was observed after one-month treatment; markedly efficacy, the ulcers/wounds where 80% to 99% of epithelial healing was observed; efficacy, the ulcers/wounds where 40% to 79% of epithelial healing was observed; inefficacy, the ulcers/wounds where less than 40% of epithelial healing was observed. If the wound was not healed at one month after treatment, the healing time was considered to be 30 days. The wound healing rate was calculated as the difference between the original wound area and the

Table 1. Baseline demographics of the patients in the two groups

Group	Age (year)	Sex ratio (M:F)	Diabetes course (y)	FBG (mmol/L)	HbA1C (%)	Hypertension history (%)	Hyperlipidemia history (%)
Control	43.7±9.8	19:19	4.5±1.1	7.2±0.8	6.6±0.6	44.7 (17/38)	31.6 (12/38)
Treatment	40.1±10.2	17:21	4.1±0.8	7.6±1.0	6.8±0.4	47.4 (18/38)	28.9 (11/38)
t/X^2	1.569	5.735	1.813	1.925	1.710	6.249	6.307
P	0.121	0.098	0.073	0.058	0.091	0.086	0.076

Note: M denotes male; F denoted female; FBG denotes Fasting blood glucose.

healed wound area divided by the original wound area, whereas the wound area was calculated by the online image recognition system.

On the 7th day of treatment, the wound secretions collected by a sterile cotton swab were cultured in the self-made enrichment broth in the enrichment culture tube. When they became turbid they were transferred to grow in a blood agar for 24 h. The bacteria in the secretions were identified using the Sceptor system for identification of (Becton Dickinson, US). The rate of positive bacterial culture was compared between the two groups.

Clinical efficacy outcomes: Wound pain: on the 7th day of treatment, the patients' pain condition was assessed using a visual analog scale (VAS), a scale ranging from 0 (painless) to 10 points (excruciating pain). Each patient was required to mark out the corresponding point on the scale according to their own pain condition. The distance from the 0-point end to the marked point was the VAS pain score.

Scar hyperplasia: at one-month follow-up after wound healing, the picture of scar hyperplasia was assessed with the use of the Vancouver scar scale (VSS), a scale covering a total score of 0 to 15 points, with higher scores indicating more severe scars, and vice versa.

Adverse reactions: at one-month follow-up after wound healing, the adverse reactions including general malaise, aggravated pain and allergic skin rash were recorded and compared between the two groups.

Detection of wound tissue growth factors: The tissues pooled from the wound of the patients in the two groups were cut into pieces, followed by ultrasound homogenization. Then the supernatant was centrifuged by high-speed centrifugation for 30 min. After that, the levels of transforming growth factor β1 (TGFβ1), insulin-like growth factor (IGF)-1, platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) in the supernatant were compared between the two groups with the use of an enzyme-linked immunosorbent assay (ELISA) according to the instructions on the ELISA kit (BD, USA).

Statistical analysis

Statistical analysis was performed with the use of the SPSS statistical software, version 17.0. The measurement data were expressed as mean ± standard deviation, and a student's *t* test was used to compare the differences between groups. The count data were expressed as rates, and measured by the X^2 test. A *P* value less than 0.05 was considered statistically significant.

Results

Baseline demographics of the patients in the two groups

There were no significant differences in baseline demographics including age, sex ratio, course of diabetes mellitus, fasting blood glucose, glycosylated hemoglobin (HbA1C) and previous internal diseases (P>0.05, **Table 1**).

Comparison of ulcer/wound healing between both groups

When compared with the control group, shorter healing time, a higher rate of wound healing, an increased overall response rate and a lower rate of positive wound culture were observed in the treatment group (All P<0.05, **Table 2**).

Comparison of clinical efficacy between the two groups

The VAS pain score and the VSS scar score were lower in the treatment group than in the control group (P<0.05). No significant difference was observed in the rate of adverse reac-

Table 2. Comparison of wound healing between the two groups

Group	Healing time (d)	Wound healing rate (%)	Overall response rate	Positive rate of wound culture (%)
Control	21.7±6.6	77.1±10.4	44.7 (17/38)	39.5 (15/38)
Treatment	16.8±5.7	84.6±12.5	60.5 (23/38)	23.7 (9/38)
t/X^2	3.464	2.843	8.482	9.201
P	0.001	0.006	0.003	0.001

Table 3. Comparison of clinical efficacy between the two groups

Group	VAS	VSS	General malaise (%)	Aggravated pain (%)	Allergic rash (%)
Control	6.9±1.8	8.9±1.0	5.3 (2/38)	21.1 (8/38)	10.5 (4/38)
Treatment	5.8±1.3	8.3±0.8	7.9 (2/38)	18.4 (7/38)	13.2 (5/38)
t/X^2	3.054	2.888	6.583	6.117	6.206
P	0.003	0.005	0.054	0.083	0.071

Note: VAS denotes visual analog scale and VSS the Vancouver scar scale.

Table 4. Comparison of clinical efficacy indicators between the two groups

Group	TGFβ1 (ng/mg)	IGF-1 (ng/mg)	PDGF (pg/mg)	EGF (pg/mgl)
Control	24.6±7.6	26.7±5.6	12.3±3.2	8.1±2.6
Treatment	31.5±9.8	33.2±6.7	16.5±3.5	12.2±3.4
t/X^2	3.430	4.589	5.460	5.905
P	0.001	0.001	0.001	0.001

Note: TGFβ1 transforming growth factor β1, IGF-1 insulin-like growth factor-1, PDGF platelet-derived growth factor, and EGF epidermal growth factor.

tions between the two groups (P>0.05, **Table 3**).

Comparison of growth factors in the wound tissues between the two groups

The levels of TGFβ1, IGF-1, PDGF and EGF in the wound tissues of the treatment group were significantly higher than those in the control group (All P<0.05, **Table 4**).

Discussion

Lower-extremity ischemic ulcer is one of the typical chronic complications in diabetic patients [1]. Studies have shown that lower-extremity vascular lesions and peripheral neuropathy attributed to insulin resistance in diabetic patients, together with lower infection resistance caused by hyperglycaemia and immunosuppression, increase their susceptibility to tissue ischemic necrosis, infection, ulcers, and even gangrene [5, 6]. Therefore, in addition to such general measures as blood glucose standards control, anti-infection, greater support, improving the resistance to ischemic ulcer wounds and wound healing has been considered as an important protocol in the treatment of lower-extremities ischemic ulcers [9]. It is reported that the repairs of ischemic ulcer wounds largely depend on mutual intervention among inflammatory cells, extracellular matrix and growth factors, of which the platelet-secreted growth factors including TGFβ1 and EGF play an essential role [10, 11]. Thus, clinicians have attempted to directly apply the platelet concentrates containing a large number of growth factors to the wound sites, expecting they could accelerate the wound healing [12].

Previous studies have demonstrated that platelet-rich plasma (PRP) containing a variety of growth factors and fibrin with high concentrations effectively upregulate the growth factor levels in the ischemic ulcers/wounds and expedite the start of repair mechanism, creating a better microenvironment for wound repairs [6, 13]. In the meantime, PRP also contains large amounts of leucocytes and monocytes, which have a favorable effect on the wound anti-infection, inhibition to staphylococcus aureus proliferation, reduction in swelling of the wound exudation, stimulation of vascular regeneration, and reduction of wound pain [14, 15]. According to international studies, multiple growth factors (including TGFβ1, IGF-1, PDGF and EGF) contained in PRP are involved in the process of healing of diabetic ischemic ulcers of lower extremity [16, 17]. Besides, as demonstrated by considerable clinical studies, PRP can promote the healing of refractory ulcers, such as diabetic foot ulcers. The working mechanisms rest largely on an increase in collagen deposition, acceleration of wound soft tissue repairs, an enhancement of early wound strength and expedition of epidermis growth [18, 19]. For more convenient clinical application, PRP is currently mixed with thrombin in proportion to calcium to form a gel-like substance, i.e. APG,

which boasts better tissue adhesion and prevention of platelet loss, assurance of growth factors with high concentrations and concentrated wounds. Furthermore, APG can also overcome the demerits of liquid reagents which are prone to evaporation and loss. As a result, APG is more suitable for clinical applications [20, 21].

In our study, APG was used to treat lower-extremity ischemic ulcers in patients with diabetes. The results showed that significantly shorter healing time, higher wound healing rate and fewer dressing times were observed in the treatment group when compared with the control group. This indicates that APG had strikingly better clinical efficacy for wound healing of lower-extremity ischemic ulcers. The causes are as follows: first, the concentrations and proportion of diverse growth factors contained in autologous PRP were physiologically similar to those of the body, to ensure better synergies among growth factors; second, the great number of growth factors helps guarantee an immune balance of inflammation and avoid immune rejection; third, APG was effective in suppressing the proliferation of bacteria (especially staphylococcus aureus), and the injury expansion. On the 7th day of treatment, the VAS score of the treatment group was significantly lower, indicating that APG had a beneficial effect on the relief of pain in lower-extremity ischemic ulcers. Moreover, at one-month follow-up after healing, the VSS score of the treatment group was significantly reduced, showing that the wound healing was improved and scar hyperplasia was not obvious. It is noteworthy that APG application did not increase the rate of various adverse reactions, suggesting better security. Further analysis showed that the levels of all the growth factors in the tissues of lower extremity ischemic ulcers were significantly higher in the treatment group than in the control group. This also supports the results of previous studies. This may be one of the major reasons why APG can promote the wound healing. Therefore, the application of APG in treating the lower extremity ischemic ulcers/wounds is effective in improving the local growth factors, promoting wound healing and the healing rate. Given the above reasons, APG is worthy of clinically extensive use.

Disclosure of conflict of interest

None.

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