Autologous platelet-rich gel for treatment of diabetic chronic refractory cutaneous ulcers: A prospective, randomized clinical trial

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The purpose of the study is to examine the safety and effectiveness of topical autologous platelet-rich gel (APG) application on facilitating the healing of diabetic chronic refractory cutaneous ulcers. The study was designed as a prospective, randomized controlled trial between January 1, 2007 and December 31, 2011. Eligible inpatients at the Diabetic Foot Care Center of West China Hospital, Sichuan University (China) were randomly prescribed with a 12-week standard treatment of ulcers (the control group) or standard treatment plus topical application APG (the APG group). The wound healing grades (primary endpoint), time to complete healing, and healing velocity within 12 weeks were monitored as short-term effectiveness measurements, while side effects were documented safety endpoints. The rates of survival and recurrence within the follow up were recorded as long-term effectiveness endpoints. Analysis on total diabetic ulcers (DUs) ($n = 117$) and subgroup analysis on diabetic foot ulcers (DFUs) ($n = 103$) were both conducted. Standard treatment plus APG treatment was statistically more effective than standard treatment ($p < 0.05$ in both total DUs and subgroup of DFUs). The subjects defined as healing grade 1 were 50/59 (84.8%) in total DUs and 41/48 (85.4%) in DFUs in the APG group compared with 40/58 (69.0%) and 37/55 (67.3%) in the control group from intent to treat population. The Kaplan-Meier time-to-healing were significantly different between the two groups ($p < 0.05$ in both total DUs and subgroup of DFUs). No side effects were identified after topical APG application. The long-term survival and recurrence rates were comparative between groups ($p > 0.05$). This study shows that topical APG application plus standard treatment is safe and quite effective on diabetic chronic refractory cutaneous ulcers, compared with standard treatment.

Diabetic ulcer (DU) is not only a serious clinical problem with negative impacts on both the life quality and survival time, but also an economic burden with significant contribution to high cost and lengthy hospitalizations. Furthermore, the nonhealing diabetic cutaneous ulcers along with the subsequent amputations may bring about costly-to-treat and painful disabilities. However, healing of the DU may be improved and most of the amputations may be prevented by more effective treatments based on diabetic education.[1]

Conventional treatments do not generally work satisfactorily for diabetic refractory ulcers. With the increasing knowledge about the pathophysiology of refractory ulcers, alterations on the local microenvironment, especially deficiency of growth factors and other bioactive substance are considered as important causes of poor healing. Biological products or emerging cellular therapies used to make up the deficiency are believed to have significantly clinical values and among them platelet-derived products have been used since 1986. Knighton et al. conducted the first clinical study demonstrating that locally applying platelet-derived wound healing factors promoted the healing of chronic refractory ulcers.[2] In 1991, Krupski et al. further reported the results of the first randomized, prospective, double-blind, placebo-controlled study on the ability of

ABI	Ankle-brachial index
APG	Autologous platelet-rich gel
APRP	Autologous platelet-rich plasma
CTGF	Connective tissue growth factor
DFU	Diabetic foot ulcer
DU	Diabetic ulcer
EFG	Epidermal growth factor
FAS	Full analysis set
GLMs	General linear models
HbA1c	Glycated hemoglobin A1c
IGF	Insulin-like growth factor
IQR	Interquartile range
ITT	Intent to treat
LOCF	Last observation carried forward
MMP	Matrix metalloproteinase
PDGF	Platelet-derived growth factor
PDWHF	Platelet-derived wound healing factor
PPS	Per-protocol set
RCT	Randomized controlled trail
S(AD)	SAD size (area, depth), sepsis, arteriopathy and denervation
SD	Standard deviation
SPSS	Statistical program for social sciences
TGF	Transforming growth factor
VEGF	Vascular endothelial growth factor

platelet factors to facilitate the healing of chronic cutaneous ulcers.[3] Over the next 20 years, several platelet-derived products have been reported to be effective as adjunctive treatments to accelerate tissue regeneration in orthopaedics, oral-maxillofacial surgery, plastic surgery, ophthalmonogy, and others.[4–6]

Autologous platelet-rich gel (APG) as a more recent platelet-derived product has been applied to clinical treatment of DUs in this century. The first prospective controlled trial on the effectiveness and safety of APG for the treatment of diabetic foot ulcers (DFUs) was conducted by Saldalamacchia et al. in 2004, providing important evidence on the significance of topical APG application on diabetic cutaneous ulcers.[7] However, it owned a small sample size of 14 and short treatment period of 5 weeks. As then, two randomized, placebo/blank-controlled trials,[8,9] with sample sizes of 72 and 42, respectively, and one randomized comparative trial[10] with 24 cases have been reported. Unfortunately, 32 cases (>20%) from the largest one of the previous four randomized controlled trails (RCTs) failed to complete the treatment or had protocol violations.[8] In 2005, we creatively found out the optimal condition for the preparation of autologous platelet-rich plasma through differential manual centrifugation and applied APG on the clinical treatment of refractory cutaneous wounds in China, reporting a complete-healing rate of 69.2% for ulcers and a higher 83.3% for sinus after APG treatment.[11] However, in this preliminary trail, we did not establish a control group. To sum up, the previous four RCTs and our preliminary trail owned relatively small sample sizes or had other shortages individually, and focused mainly on diabetic out-patients with foot ulcers (three controlled trials gave clearer declarations), though they noted positive results. Thus, there was a need for a large scale definitive RCT to determine apparent safety and effectiveness of topical APG application on chronic refractory cutaneous ulcers in the diabetic patients.

METHODS

Design

The study was designed as a prospective, randomized, controlled, and single-center clinical trial for the purpose of comparing the effectiveness of topical APG application versus standard treatment on diabetic refractory cutaneous ulcers. It was carried out at the diabetic foot care center, West China Hospital, Sichuan University (Sichuan, China) between January 1, 2007 and December 31, 2009. All eligible patients were randomized into the APG treatment group (APG group) or the standard treatment group (control group) and provided with 12-week therapies. The study protocol, patients' informed consent forms, and other study related documents were reviewed and approved by the ethics committee of the West China Hospital. This study was registered in Clinical Trials.gov (ChiCTR-TRC-09000325).

Patients

The sample size was calculated referring to the previous two RCTs reported by Saldalamacchia[7] and Driver.[8] One hundred participants were initially planned to be analyzed and one hundred twenty patients to be randomized in consideration of the potential loss rate of 20% in the study. Actually, one hundred seventeen patients were enrolled, because the screening was stopped when more than one hundred participates had finished the 12-week treatment and included in the primary analysis. The participants were randomized according to the recruitment sequence in sequentially numbered opaque sealed envelopes, through a computer-generated randomization table. One special statistical person generated the random allocation sequence, and other medical investigators enrolled the participants and assigned participants to interventions. The assessment was conducted after the patients finishing two-week ulcer standard treatments and those meeting the study inclusion criteria were allocated. Inclusion criteria were diabetic patients over 18 years of age, with at least one cutaneous ulcer, which did not improve significantly after at least 2-week ulcer standard treatments; the 2-3 Wagner's grade for the DFUs; the ankle-brachial index value ≥ 0.6 and platelet count $\geq 100{,}000$ /mm^3; no history of various drug or dressing allergies. Exclusion criteria were nondiabetic, such as malignant ulcers; diabetic acute complications such as diabetic ketoacidosis and nonketone hypertonic coma; uncontrolled systemic or local infection; severe cardiovascular, lung, liver or kidney diseases; systemic treatment medications like corticosteroids, immunosuppressive agents, as well as radiation or chemotherapy at the target sites within 3 weeks before this study; broken behavioral competence and compliance.

Treatment procedure

During the prerecruitment period, all participants received systemic therapies and standard care for the cutaneous ulcers. The former (Appendix A) consisted of intensive insulin therapy, anti-infection, nerve-trophic, and circulation-improving therapies, as well as nutritional support and anti-symptomatic treatments. Anti-hypertensive and lipid-regulating drugs were administered to the patients with hypertension and dyslipidemia, respectively. The latter was composed of topical washing, cleaning, draining, and debridement of callous and necrotic tissue, as well as dressing changes. Sequestra removal, gangrenous toe or leg amputation, and vascular reconstruction, if necessary, were carried out before recruitment. During the 12-week treatment period, systemic above-mentioned therapies continued. Ulcers of the participants in the control group were directly covered with Suile Wound Dressing (Hedonist Biochemical Technologies Co. Ltd., Taipei, Taiwan), which contained vaseline mostly and was occlusive, and then bandages, while ulcers of the APG group were treated with a topical APG application on the wound beds before a Suile baseline administration. The procedure of APG preparation was described in detail by Yuan et al.[12,13] Briefly, 20–100 mL (based on the wound sizes) peripheral venous blood was drawn into a sterilized centrifuge tube, which was mixed with 2–10 mL anticoagulant (PH 8). Following centrifugation at 313 $\times$ g for 4 minutes, erythrocyte concentrate was removed. The remaining plasma was further centrifuged at 1252 $\times$ g for 6 minutes to separate platelet-rich plasma (PRP) from platelet-poor plasma. PRP was prepared in a laboratory, and the necessary instruments and materials were a clean bench, a centrifuge, sterilized centrifuge tubes, pipettes, and sterilized pipette tips. Prepared PRP was then mixed with thrombin

and calcium gluconate in a proper proportion of 10:1 (V/V) at the bedside and trickled onto the wound bed through a three-way pipe. The Suile dressing and bandages were changed every 3 days in both groups. Generally, if the wound area reduction rates did not reach 80% or higher, or left wound areas were larger than 1 cm^2 2 weeks after APG application, repeated APG treatments were performed in the APG group.[14]

Main measurements and outcomes

Characteristics of ulcers regarding the etiology, position, size, depth, time of onset, appearance, and other related information were recorded in details at baseline. The S(AD) SAD system grading, which was designed for size (area, depth), sepsis, arteriopathy, and denervation classification,[15] was used to assess the initial healing power of the foot ulcers. Then, ulcers were examined and photographed along with dressing and bandages changes in 3-day intervals with standard light at a right angle. The images analysis and area calculation were taken with the picture-processing software ImageJ v1·46h (National Institutes of Health, Bethesda, MD).[16] The effectiveness measurements were composed of the primary wound healing grades, and the secondary time to complete healing and healing velocity. Distinguishing of different healing grades was mainly based on the "reduction rate," which was calculated as [(initial area (cm^2) − final area (cm^2)]/ initial area (cm^2),[7] at the end of the 12th week. A reduction rate of 100% was considered as healing grade 1, reduction rate of 80–99% as grade 2, 40–79% as grade 3, and 0–39% as grade 4.[11] Complete healing (reduction rate of 100%) was defined as complete epithelial cover in the absence of discharge. Apart from that, in case the wounds were suitable for skin flap transplantation (if only granulation tissue formation was enough for reconstructive plastic surgeries before the end of the 12th week) healing grade 1 was also defined. If amputation or other aggressive orthopaedic procedures were required within 12-week period, healing grade 5 was defined.[11] The area reduction rates were calculated through the digital images, while the evaluation on those who exhibited complete healing (healing grade 1), suited skin flap transplantation (healing grade 1), or required amputation or other aggressive orthopedic procedures (healing grade 5) was conducted based more on clinical examination. The healing grades assessment was conducted by a professional researcher, who was blinded to group allocation. Time to complete healing was recorded as the number of days from baseline to complete healing. Healing velocity was defined as the dynamic changes of wound area reduction rate over time at 6-day interval and calculated using the following formula: [(initial area (cm^2) − instant area (cm^2)]/ initial area (cm^2). Side effects within 12 weeks were documented as the safety endpoints. Death, hypoglycemia, decrease in hemoglobin, amputation, infection, and any serious systemic or local abnormalities were monitored for safety consideration. Phone-call or out-patient follow up was added after the treatment period until December 31, 2011. The survival of all the participants and recurrence of complete-healed ulcers were recorded and taken to evaluate the long-term effectiveness profile of the APG treatment.

Statistical analysis

If not specially mentioned, the primary measurements were performed on both the full analysis set (FAS) and per-protocol set (PPS). Apart from that, the data were analyzed with the intent to treat (ITT) principle and performed on the FAS with the last observation carried forward to impute missing values, if not mentioned. Numerical data with normal distribution were represented by mean and standard deviation (SD), or median (interquartile range [IQR]) with non-normal distribution. Demographic and clinical data were compared at baseline. Analysis on between-group differences: numerical data with normal distribution were compared by t test, otherwise, by Mann–Whitney U-test; categorical data by Chi-square test. Between-group comparison of the healing grades were carried out using the Mann–Whitney U-test, and then Kaplan-Meier curves and Log Rank (Mantel-Cox) test for survival analysis were further used to compare time to complete healing as well as the long-term survival rates and recurrence of the completely healed wounds between groups. Comparison of the wound healing velocity within and between groups was carried out by the general linear models of the repeated measures data. The safety analysis was performed on safety set. Side events were compared by Chi-square test. All tests were two-sided, and p values of 0.05 were considered significant. Statistical analysis was performed with Statistical Program for Social Sciences 17.0. Additionally, apart from the general analysis on total diabetic ulcers, a further subgroup analysis of foot ulcers was done, referring to primary endpoint, to produce more comparable and persuasive results.

RESULTS

Epidemiologic data

A total of 364 diabetic inpatients were screened in our center during this trial. One hundred seventeen patients, aged 62.8 (SD 11.6) years, with refractory cutaneous ulcers were enrolled with 59 assigned in the APG and 58 in the control group. All of them received baseline assessments and at least one kind of relevant therapy as well as consecutive ulcer measurements. A total of 14 patients asked for premature discharges from hospital, of which eight completed the data with out-patient follow-up visits, while five (three in the APG group, two in the control group) went out of contact, and one (in the control group) died of heart failure 20 days after discharge (Figure 1). Baseline data of the participants in the two groups were summarized in Table 1. The two groups were well comparable with respect to age, duration of diabetes and ulcers, as well as laboratory measurements and other relevant characteristics. In the majority (48 in the APG group, 55 in the control group) of the patients, the ulcers were located on the foot. Other ulcer locations included lower leg (six), hip (four), hand (two), neck (one), and popliteal fossa (one). The S(AD) SAD scores of DFUs were not significantly different between the APG [6 (IQR 5–8)] and the control group [6 (IQR 5–8)] ($p = 0.578$). In the APG group, 16/59 (27.1%) participants were prescribed with one time of APG treatment, 23/59 (39.0%) with two times, 11/59 (18.6%) with three times, 7/59 (11.9%) with four times, and 2/59 (3.4%) with five times within the 12-week treatment period.

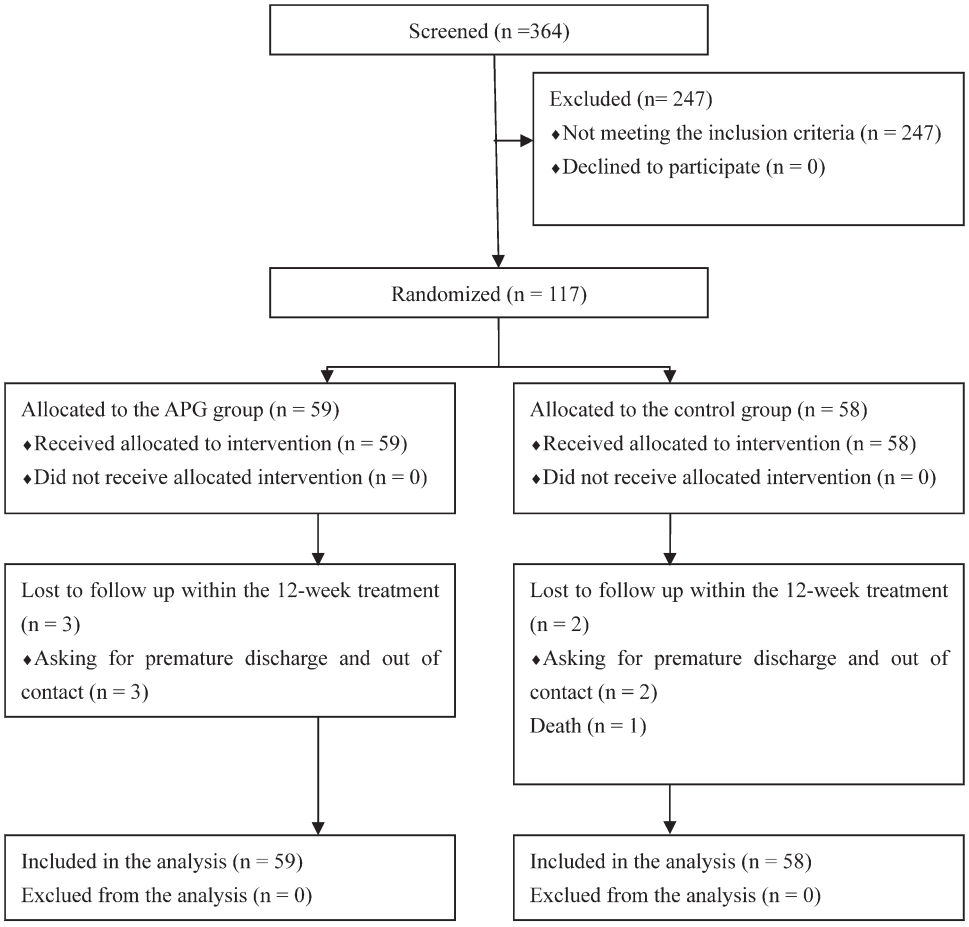

Figure 1. Flow diagram of patient disposition. Three hundred sixty-four diabetic patients with cutaneous wounds were screened. One hundred seventeen participants were randomized. Of which, 111 (94.9%) participants completed the 12-week treatment and 84 (75.7%) participants completed the long-term follow up until December 31, 2011.

Effectiveness assessment

The systematic therapies were balanced between the APG and control group (Appendix A), and the blood glucose ($p = 0.224$) and blood pressure ($p = 0.990$) levels within treatment period were comparative as well. The fasting blood glucose and two hour postprandial blood glucose were 6.8 (SD 1.8) mmol/L and 8.3 (SD 2.2) mmol/L in the APG group, and 6.7 (SD 1.8) mmol/L and 8.3 (SD 2.2) mmol/L in the control group. The systolic pressure and diastolic pressure were 130 (SD 17.8) mmHg and 77 (SD 14.7) mmHg in the APG group, and 131 (SD 21.4) mmHg and 76 (SD 11.6) mmHg in the control group.

Wound healing grades

Wound healing grades in the two groups from the ITT population were presented in Table 2. Those from PP population were replenished in Appendix B. The APG treatment plus standard care was statistically more effective than the standard care ($p = 0.026$ from ITT population, $p = 0.038$ from PP population). Of the 90 patients with complete healing defined as healing grade 1, three (in the APG group) received reconstructive plastic surgeries. Patients defined as healing grade 1 were 50/59 (84.8%) in the APG group compared with 40/58 (69.0%) in the control group from ITT population, while those defined as healing grade 1 were 50/56 (89.3%) in the APG group compared with 40/55 (72.7%) in the control group from PP population. Patients exhibiting wound area reduction rates $\geq 80\%$ (grade 1 plus grade 2) were 57/59 (96.6%) in the APG group compared with 42/58 (72.4%) in the control group from ITT population [55/56 (98.2%) vs. 41/55 (74.6%) from PP population].

The subgroup analysis of DFUs confirmed the difference ($p = 0.031$ from ITT population, $p = 0.046$ from PP population) between the two groups. Patients with DFUs defined as healing grade 1 were 41/48 (85.4%) in the APG group compared with 37/55 (67.3%) in the control group, while patients exhibiting grade 1 plus those exhibiting grade 2 were 46/48 (95.8%) in the APG group compared with 39/55 (70.9%) in the control group from ITT population. Data of DFUs from PP population were shown in Appendix B.

Time to complete healing

Kaplan-Meier time-to-healing (Figure 2) from ITT population were significantly different between the two groups [(36 (IQR 30–84) days for the APG group, 45 (IQR 18–60) days for the control group)]. The chi-square and *P* value of Log Rank (Mantel-Cox) test was 5.33 and 0.021, respectively. In addition, difference between the two groups was significant in the subgroup analysis of DFUs

Table 1. Demographics and baseline characteristics of the two groups

	APG group (n=59)	Control group (n=58)	p
Age, mean (SD), years	61.4 (13.1)	64.1 (9.4)	0.315
Gender (male/female), n (%)	37/22 (62.7)	38/20 (65.5)	0.848
Diabetes duration, median (IQR), months	90 (36–135)	120 (36–144)	0.914
Duration of ulcer, median (IQR), days	30 (15–90)	23 (14–60)	0.630
Initial wound area, median (IQR), (cm^2)	4.1 (1.4–11.4)	2.9 (1.0–10.5)	0.251
Hemoglobin, mean (SD), (g/L)	111 (19.7)	114 (25.3)	0.585
Platelet, mean (SD), (10^9/L)	216 (75.9)	196 (82.8)	0.226
Albumin/globulin, mean (SD)	1.1 (0.3)	1.1 (0.3)	0.441
Creatinine, mean (SD), (u mol/L)	88.6 (38.2)	91.1 (57.2)	0.836
HbA1c*, mean (SD), (%)	9.8 (3.1)[†]	9.8 (3.0)[‡]	0.290

The S(AD) SAD [size (area, depth), sepsis, arteriopathy and denervation] scores of diabetic foot ulcers were comparable between groups ($p = 0.578$).
*HbA1c = Glycated hemoglobin A1c.
[†]83(10) mmol/mol.
[‡]83(8.6) mmol/mol.

as well [36 (IQR 30–84) days for the APG group, 48 (IQR 18–60) days for the control group] with the Chi-Square and p value of 5.72 and 0.017, respectively.

Wound healing velocity

Wound healing velocity based on the dynamic reduction rate over time from randomization at 6-day interval was showed in Figure 3. Healing velocity displayed promotion activity of both treatments on wound healing ($p < 0.0001$), but it appeared that the APG group had a faster healing velocity than the control group ($p = 0.020$). The two groups had similar maximum median reduction rates of 100% after the 48th day.

Safety

Within the 12-week observation, no death took place in the APG group, while one participant died in the control group. Mild hypoglycemia symptoms, such as palpitation, cold sweat, perioral numbness, occurred in 5 patients of the APG group and 2 of the control group ($p = 0.438$) but alleviated soon after food intake. No severe hypoglycemia symptoms were reported. Hemoglobin did not significantly decrease after APG treatments in the APG group ($p = 0.612$). The changes of hemoglobin were not significantly different between groups ($p = 0.311$).

A total 7 cases (one in the APG group and six in the control group) were defined as healing grade 5 at endpoints. The one in the APG group received toe amputation, and another 5 of the 6 cases (one refused amputation) in the control group experienced aggressive surgical treatments (two toe amputations, two leg amputations, and one metatarsal-phalangeal joint arthrodesis). Patients defined as healing grade 5 of the two groups were not significantly different ($p = 0.061$). Infection was once not well controlled in 5 patients of the APG group, in which four relieved after further debridement and a repeated APG application (three patients reached healing grade 1 and one grade 3) and only one suffered from a surgery (grade 5) because of progressive infection. 8 patients (compared with the APG group $p = 0.558$) of the control group experienced advanced infection, in which one relived after sufficient debridement and dressing changes and reached complete healing later and the rest 7 patients (compared with the APG group $p = 0.032$) almost did not improve

Table 2. Wound healing grades for diabetic ulcers (DUs) and diabetic foot ulcers (DFUs) over time [FAS, n (%)].

	DUs			DFUs		
	APG group (n=59)	Control group (n=58)	p	APG group (n=48)	Control group (n=55)	p
Grade 1	50 (84.8%)	40 (69.0%)		41 (85.4%)	37 (67.3%)	
Grade 2	7 (11.9%)	2 (3.5%)		5 (10.4%)	2 (3.6%)	
Grade 3	1 (1.7%)	2 (3.5%)	0.026	1 (20.8%)	2 (3.6%)	0.031
Grade 4	0 (0.0%)	8 (13.8%)		0 (0.0%)	8 (14.6%)	
Grade 5	1 (1.7%)	6 (10.3%)		1 (2.1%)	6 (10.9%)	

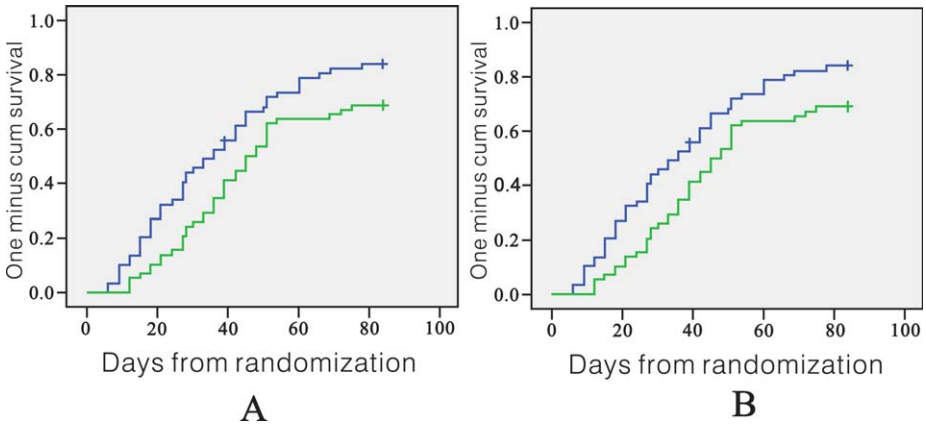

Figure 2. Kaplan-Meier time to healing (one minus cum survival) (FAS). Blue curve = the APG groups; Green curve = the control group. The A curves are for the cumulative diabetic ulcers. The Kaplan–Meier time-to-healing is significantly different between groups with the chi-Square and *p*-values of 5.33 and 0.021, and that from the foot ulcers (B) is significantly different with the chi-Square and *p* values 5.72 and 0.017.

(two were considered as healing grade 4 and five grade 5). Formication or prickling sensation was identified in 18 participants (twelve in the APG group and six in the control group, $p = 0.200$), but the symptom relived automatically soon later with the granulation tissue and epithelium of the wounds growing well.

Follow-up

It took the 3 participants, who received reconstructive plastic surgeries, 57, 80, and 85 days for a complete post-surgery closure. Five of the eight cases (in the control group), with the reduction rates of lower than 40% received APG treatments after 12 weeks of standard care and another three asked for discharge. Four cases had complete closure 9, 22, 22, and 30 days after APG treatment and 1 case developed healing grade 3 (asking for a premature discharge) 23 days after APG treatment at last observation.

Within follow up [37.5 (SD 10.2) months], 5 deaths occurred in the APG group, and 8 deaths occurred in the control group ($p = 0.394$). The time from the short-term endpoint to death was 17 (IQR 9–34) months. Ulcers recurred in 6 participants of the APG group and six of the control group. The rate of recurrence was not significantly different ($p = 0.760$ and $p = 0.179$, respectively).

DISCUSSION

Summary of the main results

In this study, topical application of APG plus standard treatment is proven to be safe and more effective on diabetic refractory cutaneous ulcers (including DFUs) compared with standard treatment. It improves the wound healing grades, shortens healing time and accelerates healing velocity, and dose not initiate general or local side effects. Meanwhile, topical APG application dose not affect the long-term rates of ulcer recurrence and survival.

Comparison and contrast of the findings

Our results are consistent with the earlier reports and more persuasive with the larger sample size and lower withdrawal rate.[7–9] The patients reaching complete healing (84.8% from ITT population and 89.3% from PP population) or exhibiting wound area reduction rate equal or more than 80% (96.6% from ITT population and 98.2% from PP population) after APG therapies are higher than those (50–85%) presented in the previous RCTs.[7–10] Of which, the highest complete healing rate of 81.3%, referring to Driver, is from PP population and after size standardization, while that before size standardization is 68.4%, and from ITT population is even a lower 32.5%. The median time of 36 days to complete healing in the APG group is shorter than the 40–80 days of the previous RCTs.[7–10] The better effectiveness could be attributed to a relatively high recovery ratio of platelets in PRP[12] and good wound-bed preparations. In addition, all of the participants in this study are inpatients, unlike the previous studies, which guarantee good compliance and adequate

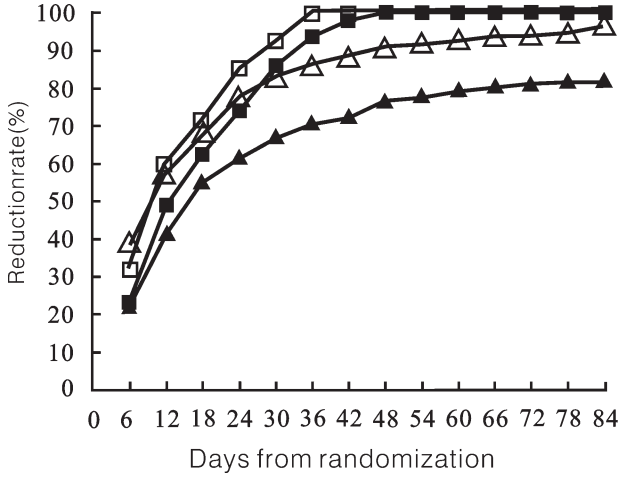

Figure 3. Curves depicting mean and median values of percentage of initial wound healed. Black triangles = mean values of the control group; white triangles = mean values of the APG group; black squares = median values of the control group; white squares = median values of the APG group. The reduction rates (on the *y*-axis) continue increasing from randomization in both of the two groups, but it heals faster in the APG than in the control group.

systematic and local treatments, including physical immobilization, wounds cleanings, debridement, draining, and regular dressing and bandages changes.

Explanations

It is generally accepted that the deficiency of growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor (TGF)-b, vascular endothelial growth factor (VEGF), connective tissue growth factor, cytokines and chemokines, and/or poor target cell responses due to hyperglycemia and diabetic vascular and neural complications are important reason for the poor healing of diabetic chronic wounds,[17] and studies have proven that the concentrations of various growth factors in wounds increased after topical APG application.[18–20] Our previous study have shown that the levels of PDGF-bb, VEGF, insulin-like growth factor (IGF)-1, epidermal growth factor (EGF), and TGF-b1 in the granulation tissues increased about twofold on the 3rd day after APG treatment, then reached peaks on the 9th day (peak of PDGF-bb was on the 3rd day) and declined to the baseline levels after 15 days.[14] These alterations of growth factors concentrations coincide with the reductions of the ulcer areas. Besides, APG's anti-bacteria effect (especially against Staphylococcus aureus),[13,21] physical support activity,[22] and its balance adjustment on the matrix metalloproteinases (MMPs) system[23,24] have been reported to have therapeutic effects. These might explain the effectiveness of APG treatment on diabetic refractory cutaneous ulcers.

Seven cases have suffered from healing grade 5. It is recorded that all the wounds are located on the feet, while the impairment of microcirculation and distal vascular diseases of the lower extremities might have resulted in poor healing. Furthermore, the wounds of the 7 patients (the baseline S(AD) SAD scores are 10 [IQR 9–10]) are more severe than other foot ulcers ($p < 0.0001$). We suggest that conservative therapies are not effective for the too severe (9 or more scoring) wounds. The bacterial culture results of the patients who have suffered from progressive infection and failed to respond to the APG treatment present the major pathogenic bacteria of Enterobacter and Candida, so we deduce that the APG treatment is not so effective to these pathogens.

Strengths and weaknesses of the study

This is the largest prospective, randomized, controlled study on the safety and effectiveness of topical APG application on diabetic refractory wounds. We enroll diabetic inpatients to guarantee the compliance as well as the balance between groups and own the lowest withdrawal rate. We prolong the observation period and creatively analyze the long-term effectiveness of the APG treatment. However, this study has certain limitations. First, this trail aims to various diabetic refractory ulcers located either on feet or not, but ulcers on lower limbs, especially those on feet, seem to be more likely to suffer from ischemia, an important cause of impaired healing. Thus, it is difficult for us to evaluate the healing ability of all the ulcers by the same criteria. To make up this deficiency, we have analyzed the most important general factors involved in wound healing, and made a further subgroup analysis of DUFs based on an baseline evaluation according to S(SAD) SAD score system to guarantee the between-group comparability. Second, 14 participants (eight complete the follow-up visits) have asked for premature discharges from hospital within the 12-week period. Thus, the potential noncompliance might influence the effectiveness of treatments and disturb balance between groups. Third, we define wound healing as complete epithelial cover, while it is increasingly usual for the definition of healing to include maintenance of healing for at least 2 weeks. Accordingly, the judgment on wound healing might be misguided for missing the data of those who have suffered from early wound breakdown. Fortunately, the follow-up data shows that all the wound breakdowns (recurrence) occur 2 weeks after completely healed (either located on feet or not) in the study. A more proper and less debatable definition is suggested to be brought out in the future. Fourth, calculation on wound areas by the picture-processing software image J is accurate and nonpolluting compared with the traditional transparency tracing method.[25,26] However, both of the two methods above might be more suitable for flat wounds, because changes of wound area could not fully reflect the healing grades for sinuses or wounds with large dead space. In these circumstances, medium packing method is more recommended.[25] Nevertheless, the medium (usually selected as saline) might influence the absorption of bioactive factors and reduce the effectiveness of APG treatment, so we have not considered it. Fifth, owning to some ethical and operational reasons, it can not be designed as a double-blinded trail, which might reduce the argumentation intensity of the data.

Conclusion, implications, and policy

In summary, this prospective, randomized, controlled study indicates that topical APG application on standard treatment is safe and effective in treating diabetic chronic refractory cutaneous ulcers. Topical application of APG improves the wound healing grades, shortens the healing time and accelerates the healing velocity. Beside, the manual preparation of APG seems to consume few and easy to learn. These findings along with those in our preliminary studies help a lot to popularize this manually prepared APG in the treatment of refractory wounds and thus to overcome the challenge from them for us mankind. In the future, double-blinded trails and basic studies involving the mechanism, such as the role of MMPs on the healing-promoting action of APG (which our team is now focusing on), are needed. Furthermore, we should make some efforts to explore more biological stuff for the treatment of refractory wounds.

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APPENDIX A

Table A1. Major systematic drugs included in each group

		APG group	Control group
Anti-diabetic drugs	Insulin	Recombinant human insulin injection, recombinant human insulin lispro injection, insulin aspart injection, isophane protamine recombinant human insulin injection, and insulin glargine injection	Recombinant human insulin injection, recombinant human insulin lispro injection, insulin aspart injection, isophane protamine recombinant human insulin injection, and insulin glargine injection
	Oral anti-diabetic drugs	Metformin hydrochioride tablets, acarbose tablets, and rosiglitazone maleate tablets	Metformin hydrochioride tablets, acarbose tablets, and rosiglitazone maleate tablets
Anti-hypertensive drugs	Calcium channel blockers (CCBs)	Nifedipine controlled release tablets, amlodipine besylate tablets, and felodipine tablets.	Nifedipine controlled release tablets, amlodipine besylate tablets, lacidipine tablets, and felodipine tablets.
	Angiotension converting enzyme inhibitors / Angiotensin II receptor blocker s (ACEIs/ARBs)	Irbesartan tablets, sodium fosinopril tablets, perindopril tablets.	Irbesartan tablets, sodium fosinopril tablets, perindopril tablets
	Duritics	Spironolactone tablets, hydrochlorothiazide tablets, and indapamide tablets.	Spironolactone tablets, hydrochlorothiazide tablets, and indapamide tablets.
Lipid-regulating drugs	Statins	Simvastatin tablets, and pravastatin sodium tablets	Simvastatin tablets, and pravastatin sodium tablets
	Fibrates	Fenofibrate capsules.	Fenofibrate capsules.
anti-infectious drugs	nitroimidazoles	Tinidazole and glucose injection, and metronidazole injection.	Tinidazole and glucose injection, and metronidazole injection.
	Quinolones	Levofloxacin lactate and sodium chloride injection, ciprofloxacin and sodium chloride injection, and moxifloxacin injection.	Levofloxacin lactate and sodium chloride injection, ciprofloxacin and sodium chloride injection, moxifloxacin injection, and gatifloxacin injection
	Penicillins	Amoxicillin sodium and sulbactam sodium for injection, oxacillin sodium for injection,	Amoxicillin sodium and sulbactam sodium for injection, oxacillin sodium for injection,

Table A1. Continued.

		APG group	Control group
		benzylpenicillin sodium for injection, and piperacillin sodium and tazobactam sodium for injection.	benzylpenicillin sodium for injection, and piperacillin sodium and tazobactam sodium for injection.
	Cephalosporins	Ceftazidime for injection, cefoperazone sodium and sulbactam sodium for injection, cefuroxime sodium for injection, cefaclor for oral suspension., cefoxitin sodium for injection.	Ceftazidime for injection, cefoperazone sodium and sulbactam sodium for injection, cefuroxime sodium for injection, cefaclor for oral suspension., cefoxitin sodium for injection, and ceftriaxone sodium for injection.
	Glycopeptides	Vancomycin hydrochloride for injection.	Vancomycin hydrochloride for injection.
	Carbapenems	Imipenem and cilastatin sodium for injection.	Imipenem and cilastatin sodium for injection.
	Aminoglycosides	Amikacin sulfate injection	Amikacin sulfate injection
	Macrolides	Azithromycin lactobionate for injection	Azithromycin lactobionate for injection
	Lincomycins	Clindamycin phosphate for injection	Clindamycin phosphate for injection
	Anti-fungal agents	Fluconazole injection, and nystatin tablets	Fluconazole injection, and nystatin tablets
Nerve-trophic drugs		Mecobalamin injection, and citicoline sodium and sodium chloride injection	Mecobalamin injection, and citicoline sodium and sodium chloride injection
Circulation-improving drugs	Anti-platelet agents	Aspirin enteric-coated tablets, and clopidogrel tablets	Aspirin enteric-coated tablets, and clopidogrel tablets
	Anti-coagulants	Low molecular weight heparin calcium injection, and warfarin tablets	Low molecular weight heparin calcium injection, and warfarin tablets
	Vasodilators	Alprostadil injection, and cilostazol tablets	Alprostadil injection, and cilostazol tablets
	Medicines to improve venous function	Diosmin tablets, sulodexide injection	Diosmin tablets, sulodexide injection
	Others	Shuxuetong injection, and salvia miltiorrhiza injection	Shuxuetong injection, and salvia miltiorrhiza injection

APPENDIX B

Table B1. Wound healing grades for DUs and DFUs over time [per-protocol set, *n* (%)]

	DUs			DFUs		
	APG group (*n*=56)	Control group (*n*=55)	*p*	APG group (*n*=46)	Control group (*n*=52)	*p*
Grade 1	50 (89.3%)	40 (72.7%)		41 (89.1%)	37 (71.2%)	
Grade 2	5 (8.9%)	1 (1.8%)		3 (6.5%)	1 (1.9%)	
Grade 3	0 (0.0%)	1 (1.8%)	0.038	1 (2.2%)	1 (1.9%)	0.046
Grade 4	0 (0.0%)	7 (12.7%)		0 (0.0%)	7 (13.5%)	
Grade 5	1 (1.8%)	6 (10.9%)		1 (2.2%)	6 (11.5%)	