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Original Article

A study on the therapeutic effects of autologous platelet-rich plasma in the treatment of limbsrefractory wound infection

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Abstract: Objective: To discuss the therapeutic effects of autologous platelet-rich gel (APG) in the treatment of limbs refractory wound infection. Methods: A total of sixty patients with refractory wound infection in lower limb weretreated in our hospital from January 2016 to December 2016, who had received one month of wound treatment but unhealed. After random grouping, thirty of them were treated with APG as the treatment group (Group APG), the rest thirty were treated with isotonic saline as the control group (Group Con). The wound areas, pain scores and colony-forming units (CFU) on infected tissue of the patients in the two groups on admission and those at the 7th day after treatment,the complications on the 7th day after therapy and thr follow-up cure time of patients were compared and analyzed. Results: The wound areas in Group APG at the 7th day after therapy were evidently declined compared with those on admission (P=0.021). At the same time, the wound areas of Group APG were smaller than those of Group Con (P=0.035). The wound CFU of two groups were both decreased gradually from the 1st to the 7th day after treatment (all P<0.05). Besides, the decline in Group APG was sharper than in Group Con (P=0.003). There was no statistical difference in patients' wound pain scores in two groups. The cure time of Group APG was 15.2±2.2 days, while that of Group Con was 20.2±2.2 days, and the differencehad statistical significance (P=0.032). The wound infection rate (P<0.001) and scar index (P=0.049) of Group APG after treatment were apparently lower than those of Group Con. Conclusion: Autologous platelet-rich gel can decrease wound areas, reduce CFU on wound, shorten the wound cure time and lessen the occurrence of complications.

Keywords: Autologous platelet-rich gel, refractory wound infection, lower limb

Introduction

The refractory infected wound caused by wound infection has increased by years in China and its incidence rate was increased from 48% to 67.5% in 2015 [1, 2]. The traditional treatment for wound infection included debridement, application of negative pressure vacuum extraction, topical application of antibiotics, medicine for promoting epidermis growth, change fresh dressing for a wound, etc. However, all the above treatment measures have relatively little influence on the direct healing process of wound [3, 4].

Refractory wound infectiondoes not threaten patients' life such as sepsis, etc., but the patients with refractory wound infection still need long-term dressing change and antibiotics application, which will then lead to drug resistance in patients to various strains, thus significantly enhancing the difficulties in wound infection treatment, increasing hospitalization and the expenses [5, 6]. Some patients even suffered from severe complications and sequelae, which could result in a significant decrease in life quality [2]. Consequently, the therapy for refractory wound infection has been an urgent need.

In recent years, autologous platelet-rich gel (APG) has been a research hot spot. It was found that APG was able to repair bone and soft tissue injuries, inhibit the growth of bacterial, reduce wound infection and so forth [7-9]. Therefore, in this study, 60 patients with lower limb refractory wound infection were collected, 30 patients were treated with APG and the rest 30 patients were treated with isotonic saline. Through observing wound areas, all the colony-

forming units (CFU) on wound tissue, pain scores of wound, cure time and complications of wound infection of patients, the antibacterial effect and healed-promoting effect for refractory wound infection of APG could be evaluated.

Materials and methods

This clinical trial was approved by Ethnic Committee with signed informed consent from all the patients.

Cases collecting

A total of sixty patients with refractory wound infection of lower limb, who had received one-month wound treatment but unhealed, were treated in our hospital from January 2016 to December 2016.

Inclusion criteria: Patients who met the diagnosis criteria of refractory wound infection withoutany sign of improvement after one month of debridement, application of negative pressure vacuum extraction, application of antibiotics, change fresh dressing for a wound, etc.; patients whose wound appeared white skin edge, darkened necrotic tissue, little and atrophic granulation tissue and sometimes accompanied with exposure of skeleton, tendon or muscle tissue; patients aged 18-80 years old; patients who were not treated with APG or other similar therapies before.

Exclusion criteria: Patient who had severe cardiac, liver, lung, kidney or other systematic diseases; patient who had generalized infection or severe complications; patients who were pregnant or in lactation; patients without limbs refractory wound infection.

Grouping

According to random number table, patients were divided into two groups with 30 cases in each: one group was treated with APG (Group APG); the other group were treated with conventional isotonic saline as the control group (Group Con).

Treatment measures

The wounds of patients in Group APG were covered with APG wet dressings and then totally exposed (change the dressing once per day). While, the wounds of patients in Group Con were only covered with isotonic saline gauzes and then a half of the wound was exposed (change the dressing once per day).

Preparation of APG: The preparation process of APG was carried out in strict accordance with aseptic procedures. The venous blood (40 mL) was collected from every patient, and then put into the 50-mL centrifuge tube with 4 mL of sodium citrate added in advance. After gently shaking, it was centrifuged at 2000 rpm/min for 10 min. All the upper plasma (red blood cells layer) which was 1 mm above the interface was absorbed and transferred to a new centrifuged tube and re-centrifuged at 4000 rpm/min for 10 min. The upper plasma was discarded and the left fluid was the platelet-rich plasma. Next, the platelet-rich plasma was mixed up with 10% $CaCl_2$ in proportion of 1:9. Finally, APG was obtained by this way and preserved at -20°C [10, 11].

Follow-up

Change of wound areas: Homogeneous paper from CompatDP was applied to cut as shape as the patients' wound and then weighted by the electronic balances with the accuracies of 0.001 g and 0.0001 g respectively. The weight of the wound shape-like paper represented the wound areas, to be more specific, the heavier the wound shape-like paperswere, the bigger the wound areaswere. Patients' wound areas of both groups were measured respectively at the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, and 7^{th} day after treatment.

CFU in vitro: The infection wound tissues were taken respectively at the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, and 7^{th} day after treatment, coated on LB solid medium (10 g/L tryptone, 5 g/L yeast extract and 10 g/L sodium chloride) and incubated in the constant temperature incubator at 37°C for 12 h. Then the CFU was counted, and its everyday means and standard deviations of both groups were calculated [12].

Wound tissue pain scores: Both groups of patients' pain scores were evaluated respectively by visual analogue scale on admission and at the 7^{th} day after corresponding treatment (0 point means painless, 10 points means extremely painful). The wound tissue pain scores of all patients in two groups were recorded [13].

Table 1. Patients' general information

Item	Group APG (n=30)	Group Con (n=30)	*P* value
Male/female	25/5	20/10	0.136
Age	45.5±5.6	48.1±8.2	0.674
Primary diseases			
Tibial and fibular fracture	7	8	0.766
Calcaneus fracture	5	10	0.136
Lower limb open fracture	9	6	0.371
Tibia osteomyelitis	2	3	0.640
Femur osteomyelitis	3	2	0.640
Infection after foot orthopedic surgery	4	1	0.161
Diabetes	3	2	0.640

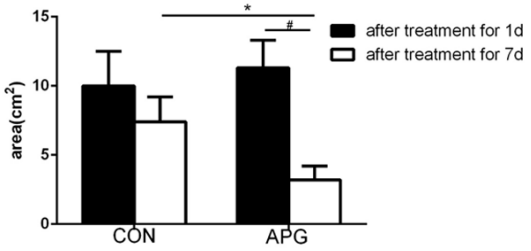

Figure 1. Comparison of patients' wound areas on admission and at the 7th day after the treatment in two groups. *P<0.05 (the 1st day vs. the 7th day after the treatment of Group APG); #P<0.05 (Group APG vs. Group Con at the 7th day after the treatment); CON, Group Con; APG, Group APG.

Cure time and complications: All the patients were admitted to our hospital for return visits or therapies at the 1st, 2nd, 3rd and 4th month in accordance with prior agreement. All patients' cure time, wound infection rate, scar index, joint deformity and other complications were recorded.

Observation indexes

Primary observation indexes: Wound areas, CFU count and wound pain scores. Secondary observation indexes: Wound cure time, complications of wound infection.

Statistical analysis

All measurement data were expressed as mean ± standard deviation (mean ± sd), and analyzed by SPSS 17.0 software package. The difference value before and after the treatment as well as the baseline before treatment and the value after treatment between groups were compared by one-way ANOVA. Comparison of the data before and after the treatment within group was conducted by paired t test. Comparison of two groups of patients' wound CFU in 7 days after treatment at multiple time points were underwent by repeated measures ANOVA, combined with Bonferroni correction. Comparison of complications between two groups was conducted by t test. GraphPad Prism 5.0 was applied for analysis and mapping of all the graphs. Inspection level: α=0.05. When P< 0.05, the difference was statistically significant.

Results

Patients' general information on admission

There was no statistical difference in patients' sex ratio, age and primary diseases (**Table 1**). Patients' primary diseases included 15 cases of tibial and fibular fractures, 15 cases of calcaneus fractures, 15 cases of lower limb open fractures, 5 cases of tibia osteomyelitis, 5 cases of femur osteomyelitis, 5 cases of infection after foot orthopedic surgeries and 5 cases of diabetes.

Comparison of patients' wound areas on admission and at the 7th day after the treatment in two groups

Among the patients with limbs refractory wound infection, those in Group APG had an obvious decline in wound areas at the 7th day after the treatment. Compared with those on admission, this difference had statistical significance (P=0.021). At the meantime, wound areas of patients in Group APG at the 7th day after the treatmentwere apparently less than those in Group Con (P=0.035). As for Group Con, there was no statistical significance in wound areas between on admission and at the 7th day after the treatment (P=0.082). See **Figure 1**.

Comparison of patients' 7-day wound CFU after the treatment in two groups

Among the patients with limbs refractory wound infection, their wound CFU in Group APG and Group Con both gradually reduced from the 1st

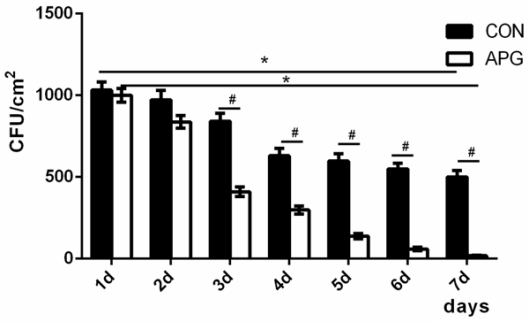

Figure 2. Comparison of patients' 7-day wound CFU after the treatment in two groups. *P<0.05 (comparison of the 7th and 1st day after treatments of Group APG and Group Con); #P<0.05 (comparison between Group APG and Group Con after the treatment); CON, Group Con; APG, Group APG.

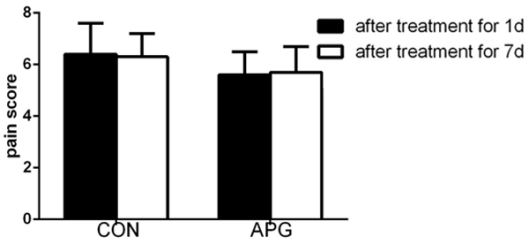

Figure 3. Comparison of patients' wound pain scores between on admission and at the 7th day after the treatment. CON, Group Con; APG, Group APG.

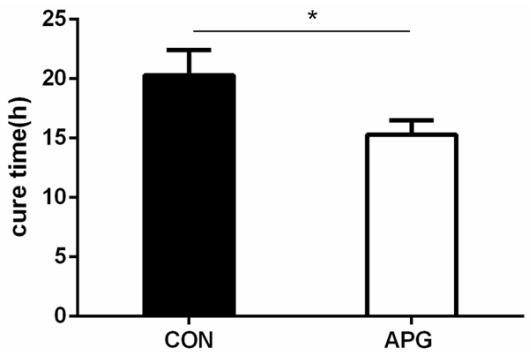

Figure 4. Comparison of patients' cure time in two groups. CON, Group Con; APG, Group APG; *P<0.05, the cure time of two groups was statistically different.

to 7th day after their corresponding treatments (all P<0.001). Moreover, the decline of Group APG was sharper than that of Group Con (P=0.003). Compared with the 1st day after the treatment in Group APG, wound CFU was clearly lessened at the 7th day after the treatment (P<0.001). The comparison of patients' wound CFU at the 7th day after the treatment in two groups indicated that the wound CFU count of Group APG was much less than that of Group Con (P=0.003). There was no statistical difference in wound CFU at the 1st and 2nd after the treatment in both groups (P=0.812, 0.634). See **Figure 2**.

Comparison of patients' wound pain scores between on admission and at the 7th day after the treatment

There was no statistical difference in patients' wound pain scores between on admission and at the 7th day after the treatment in Group APG (P=0.936) and in Group Con (P=0.90). As for the change of patients' wound pain scores before and after treatment, there was no statistical difference between Group APG and Group Con (P=0.892). See **Figure 3**.

Comparison of patients' cure time in two groups

Four-month follow-up was carried out for patients in both groups without any missing. All the patients were admitted to our hospital as agreed before at the 1st, 2nd, 3rd and 4th month after treatment for return visits or therapies and the patients' cure time was recorded. The cure time of Group APG was 15.2±2.2 days, while that of Group Con was 20.2±2.2 days. So, the cure time of Group APG was apparently shorter than that of Group Con and the difference was statistically significant (P=0.032). See **Figure 4**.

Comparison of patients' complications in two groups

All the sixty patients included in this clinical research finished the four-month follow-up without any missing. And all of them had return visits or therapies at the 1st, 2nd, 3rd and 4th month after discharge. Consequently, their complications, wound infection rate, scar index and joint deformity were recorded. The results showed that both the wound infection rates and scar indexes of Group APG were obviously lower than those of Group Con and the differenceswere statistical (P<0.05). Nevertheless, the incidences of joint deformity of both groups were lower and there was no statistical difference (**Table 2**).

Table 2. Complications of patients in two groups

	Group APG	Group Con	t value	*P* value
Infection rate	3.4±0.5	20.4±2.3	-12.51	0.000
Scar index	4.6±1.5	9.3±2.5	-2.792	0.049
Joint deformity	1.3±0.2	1.6±0.2	-1.837	0.140

Discussion

APG is a kind of concentrated platelet gel obtained from two rounds of centrifugation of autologous blood, and the massive concentrated platelet can release a great quantity of growth factors after activation, such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor-β (TGF-β), vascular endothelial cell growth factor (VEGF) and so forth [14]. Through chemotaxis, constriction of blood vessels and inducing the proliferation of epithelial and endothelial cells, PDGF can regulate wound repair by secretion of TGF-β [15].

EGF is a kind of cell division promoting factor. On the one hand, it can stimulate the proliferation and differentiation of collagenocyte, and facilitate the re-epithelialization of wounds. On the other hand, it can promote the formation of blood vessels [15]. VEGF is a kind of platelet-derived factor, which can stimulate the regeneration of vascular endothelial cell and the formation of cell-extracellular matrix, to accelerate the repair of wounds [16].

In this study, the APG prepared by centrifugation was used in the treatment of limbs refractory wound infection. Compared withthe Group Con, APG could obviously lessen the infected wound areas. Chen et al. discovered that APG could significantly speed up the wound cure process of children's facial second degree burn and shorten the cure time [10]. This founding was consistent with our study, which indicated that APG might facilitate the wound cure through the above growth factors.

This experiment found that the APG used in the treatment of refractory wound infection could reduce the CFU on infected wounds and accelerate wound cure. It was in line with Yang's founding that APG was able to inhibit the reproduction of staphylococcus aureus and thus reducing wound infection rates [17]. The high concentrations of platelet and white blood cell in APG were the key to exert its antibacterial effects. The white blood cells, such as neutrophil, mononuclear, macrophage, etc., play vital roles in innate immune response, antimicrobial immune response and other defense responses. The platelet could activate a large number of growth factors that could constitute a complex regulatory network, which could not only accelerated wound tissue repair but also enhanced local anti-inflammatory ability. Bielecki et al. held the view that after the APG were released, there would be massive antibacterial, anti-inflammatory substances and other metabolites like superoxide. They could directly kill bacteria and accelerate other immune cells to indirectly kill bacteria [18]. As a result, APG could play a better role in preventing infection [19].

APG is an effective autologous component with low incidence of immune rejection. In the absence of external infection sources, the incidence of blood borne disease was extremely low [20]. At the same time, APG is concentrated from the platelet, so it can promote the adhesion of transplanted heterogeneous acellular dermal matrix and the wound base, avoid sliding, eliminate partial cavity and reduce infection witha hemostatic effect [21]. Kim et al. reported that one patient suffered obvious pain and swelling after APG treatment in his study, but the above symptoms disappeared two days later. However, there was no any discomfort occurring to other patients [22]. During the four-month follow-up, our study found that patients treated with APG had apparently less complications compared with the Group Con. Nevertheless, we should also pay attention to the adverse reactions such as the pain and swelling on the wound site. Once the adverse reaction occurred, the treatment must be terminated and the corresponding measures need to be taken to recover the local infected wounds and restore systemic functions.

There were a few limitations in our study. Firstly, as the requirements for APG preparation are very strict, and the platelet can be activated easily, which may lead to the failure of the preparation. It is a waste of autologous blood and the energy of the experimenter. Secondly, perhaps the purity of the prepared APG is not high enough to achieve the satisfying therapeutic effects for some patients. Thirdly, the sample

size of this study was limited, so a further experiment with a larger sample size will be carried on later to enhance the credibility.

In conclusion, autologous platelet-rich gel can decrease wound areas, reduce CFU on wound, shorten the wound cure time and lessen the occurrence of complications, which is worth of a dissemination trail in clinic.

Disclosure of conflict of interest

None.

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