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Infected bone inactivation combined with transplantation of autologous platelet-rich plasma and bone marrow for treatment of chronic osteomyelitis

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Abstract. – OBJECTIVE: Here we tested the therapeutic efficacy of infected bone inactivation combined with transplantation of autologous platelet-rich plasma and bone marrow in chronic osteomyelitis.

PATIENTS AND METHODS: 64 patients with chronic osteomyelitis were randomly divided into two groups. Patients in control group received conventional antibiotic and surgical treatments, while patients in the experimental treatment group underwent infected bone inactivation combined with transplantation of autologous platelet-rich plasma and bone marrow. The X-ray, histological, and biochemical (alkaline phosphatase) changes were assessed at 4, 8, 12 and 16 weeks after the treatment.

RESULTS: At all tested study points, X-ray and histological scores, and alkaline phosphatase levels were significantly better in patients of the experimental treatment group.

CONCLUSIONS: Infected bone inactivation combined with transplantation of autologous platelet-rich plasma and bone marrow achieves beneficial therapeutic results in chronic osteomyelitis.

Key Words:
Orthotopic transplantation, Autologous platelet-rich plasma, Bone marrow, Chronic osteomyelitis.

Introduction

Chronic osteomyelitis is a long-term disease with high rates of surgery failure, infection and reoccurrence. Pathophysiology of this disease is complex[1,2]. While antibiotics have some therapeutic effects, the treatment of chronic osteomyelitis remains difficult because of high vulnerability to infections with methicillin-resistant *Staphylococcus aureus* (MRSA)[3-7]. Delayed treatment adversely affects appearance and function of the affected limb, and complications (fracture, joint stiffness, deformity, and occasional cancer) aggravate and threaten patient life. Autologous transplantation of platelet-rich plasma combined with bone marrow has been effective in treating spinal fusion and bone non-union. This therapy promotes formation of the callus at the fracture end and is beneficial for healing of the fracture. However, similar studies in chronic osteomyelitis are rare[8-10]. We tested therapeutic efficacy of infected bone inactivation combined with autologous platelet-rich plasma and bone marrow transplantation in 32 patients with chronic osteomyelitis. In the present report, we demonstrate beneficial effects of this treatment.

Patients and Methods

Patients

Sixty-four patients with chronic osteomyelitis who were admitted to Xi'an Honghui Hospital from April 2010 through April 2013 were enrolled and randomly divided into two study groups. All patients had various degrees of inflammatory dead space and/or sequestrum, and prolonged unhealed sinus tract. Patients were informed about advantages and disadvantages of suggested treatments, agreed to participate in the study, and signed informed consent forms. The study protocol was approved by the Ethics Committee of our Hospital. Characteristics of study patients are shown in Tables I and II. Both study

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groups were comparable for studied clinical characteristics. We made diagnoses based on international criteria, such as formation of chronic sinus tract, limb pain, swelling of the sinus tract, and secondary ulcer. All symptoms and manifestations were confirmed by imageological examination.

Methods

Bacteria from skin lesions were cultured before the surgery to test for pathogen antibiotic sensitivity and antibiotics were administered accordingly. Patients' nutritional status was rectified. Patients with no apparent sequestrum or accidental symptoms received drug treatment and hot compress therapy.

Patients in control group received conventional pharmacological and surgical treatments. The sinus tract at the lesion region was incised, a window was made in the bone, and bone lesion was cleaned and rinsed. Then, artificial bone (Beijing Yikang Biotechnology Development Center, Beijing, China) supplemented with antibiotics was implanted. To cover the big cavity remaining after lesion cleansing, a piece of autologous iliac bone was obtained and cut into granules. The granules were immersed in normal saline combined with antibiotics and mixed with artificial bone to form a "sandwich" for transplantation. In this "sandwich", one layer was made of artificial bone and another layer of autologous bone. Skin was closed by primary suture or transfer of a skin flap. After lesion clearance, patients with high fracture risk received external fixation or plaster immobilization. Overarticular fixation was applied for less than 6 weeks. Usually, a drain tube was inserted after the primary suture, and appropriate antibiotics were routinely applied after the surgery. The drain tube was removed when the drainage was less than 5 ml daily.

Patients of the experimental treatment group received a similar treatment as control patients. In addition, these patients underwent an infected bone inactivation and orthotopic transplantation of autologous platelet-rich plasma combined with bone marrow. To prepare platelet-rich plasma, we aseptically obtained 200 ml of venous blood in a centrifuge tube with 10% sodium citrate. After mixing, blood was subjected to centrifugation for 10 min. After centrifugation, plasma and the upper 1-mm layer of red blood cells were transferred into a new centrifuge tube and underwent a second centrifugation. This divided plasma into platelet-deprived and platelet-rich fractions. The former fraction was discarded, while the latter fraction was mixed. To prepare concentrated preparation of autologous bone marrow, a puncture needle was used to extract bone marrow from posterior superior iliac spine under aseptic condition. Fifty ml of bone marrow were extracted and added to an aseptic tube for subsequent 10-min centrifugation. After centrifugation, the supernatant was discarded, while concentrated bone marrow was kept. Platelet-rich plasma and bone marrow were mixed prior to transplantation. The transplantation was applied after removing the dead and/or infected bone from the area that was the closest to the lesion. First, a pulsed hydraulic gun was used to flush the affected region and aspirate infected tissue. Then, a mixture of autologous platelet-rich plasma and bone marrow was injected 1 time/week for the total of 3 weeks.

Therapeutic Effect Evaluation

Therapeutic effect was evaluated according to the following criteria. On weeks 4, 8, 12 and 16 after the surgery, modified Gary X-ray scoring criteria were applied to evaluate bone repairing. A 25% callus was ranked as 0 points, while calluses of 26%-50%, 51%-75%, and 76%-100% were respectively ranked 1, 2, and 3 points.

In bone union evaluation, nonunion was ranked as 0 points, while un-confirmation, partial union, confirmed union were respectively defined as 1, 2, and 3 points.

We also carried out histological examinations. Samples of transplanted bone and osteotomy space were collected and fixed in 4% paraformaldehyde for 24 hours, decalcified in 10% EDTA, gradually dehydrated, and waxed

Figure 1. Lesion appearance before the treatment.

Figure 2. Lesion appearance after the treatment.

prior to observation under light microscope. No callus formation was ranked as 0 points, formation of a small callus was defined as 1 point, partial formation as 2 points, callus filling the lesion as 3 points, and bone union as 4 points.

Types of connection between the graft and bone tissue were assessed as nonunion (0 points), fibrous union (1 point), cartilage union (2 points), bone union (3 points), and complete bone union (4 points).

In addition to the above examinations, 4 ml of venous blood were collected for analysis of serum alkaline phosphatase (AKP). The assay was done using SYNCHRON CX9 PRO automatic biochemistry analyzer (Beckman-Coulter Inc., Brea, CA, USA).

Statistical Analysis

The SPSS13.0 statistical software was used for the analysis. Quantitative data were expressed as mean ± SD and compared using the *t* test, while qualitative data were analyzed by the chi-square test for univariate analysis. Differences at a $p < 0.05$ were considered as statistically significant.

Results

At all tested time points (4, 8, 12 and 16 weeks after the treatment), the X-ray scores were significantly higher in the experimental treatment group (Table III).

We next compared histological scores between study groups. Similar to X-ray scores, histological scores were significantly higher in the experimental treatment group (Table IV). Respective images before the surgery are shown in Figures 1 and 2, and after the surgery in left and right panels of Figure 3.

Finally, we tested AKP levels in study groups. At 4, 8, 12 and 16 weeks after the treatment, AKP levels were significantly higher in the experimental treatment group (Table V).

On histological examination at 4 weeks after the treatment, growth of fibrous connective tissue in transplanted bone and osteotomy section, and formation of sporadic cartilage cell cluster were evident. At 8 weeks, there was vessel growth in transplanted bone and osteotomy section, sporadic bone trabecula, and abundant cartilage cell cluster. At 12 weeks post-treatment, we

Figure 3. Lesion appearance after the treatment.

Table I. Clinical characteristics of study patients.

Characteristics		Control group (n = 32)	Experimental treatment group (n = 32)
Gender, male/female		22/10	20/12
Age, year		31.7 ± 3.4	31.9 ± 3.5
Disease course, month		4.7 ± 1.3	4.5 ± 1.2
Affected area	Tibia and fibula	14	16
	Radius and ulna	12	10
	Other locations	6	6

Footnote: Data are presented as mean ± SD or as absolute numbers. There were no significant differences between two groups.

Table II. Pathogenic bacteria in study patients.

Pathogenic bacteria	Control group (44 strains)	Experimental group (46 strains)
Staphylococcus aureus	18	20
Escherichia coli	10	8
Group B Streptococcus	8	10
Streptococcus pneumoniae	4	2
Other pathogens	4	6

Footnote: Data are presented as absolute numbers. There were no significant differences between two groups.

observed formation of abundant vessels, mature bone trabecula, and cartilage tissue. Finally, at 16 weeks after the treatment, the vessel structure in transplanted bone and osteotomy space was decreased or disappeared, there were many mature bone trabecula, and bone marrow cavity was partially or completely recanalised.

Discussion

Chronic osteomyelitis leads tissue necrosis, poor microcirculation, lower production of local growth factors, and less efficient self-repair. This may be related to cell apoptosis or changes in receptor expression on cell membrane[11,12]. Several therapeutic methods for chronic osteomyelitis exist[13-15]. Lesion clearance and drainage can be applied to remove the sinus tract, scar tissue, dead bone, and foreign bodies. However, this method exhibits poor efficacy with regard to large lesions, with long healing times and unstable therapeutic effects[16]. One can resort to limb amputation if the infection can no longer be controlled. While this method radically treats the disease, it also causes serious sequelae and majorly affect the patient quality of life[17].

Platelet-rich plasma contains high levels of various grow factors, including the platelet-derived, transforming, epidermal, fibroblast, and insulin-like growth factors. These promote differentiation of repairing cells[18,19]. Thus, platelet-derived growth factor increases the growth of granulation tissue, transforming growth factor promotes migration of epidermis cells, composition and moulding of the extracellular matrix, epidermal growth factor stimulates mitosis and cell proliferation, while fibroblast growth factor facilitates regeneration of epidermis and endothelium[20,21]. In addition, platelet-rich plasma

Table III. X-Ray findings in study patients.

Group	Post-treatment, weeks			
	4	8	12	16
Control group (n = 32)	1.02 ± 0.53	1.24 ± 0.61	2.37 ± 0.45	2.87 ± 0.54
Experimental treatment group (n = 32)	1.85 ± 0.32	2.97 ± 0.43	3.82 ± 0.52	4.55 ± 0.39
p	< 0.05	< 0.05	< 0.05	< 0.05

Footnote: Data are presented as mean ± SD.

Table IV. Histological scores in study patients.

Group	Post-treatment, weeks			
	4	8	12	16
Control group (n = 32)	1.13 ± 0.45	2.04 ± 0.71	3.12 ± 0.62	3.43 ± 0.33
Experimental treatment group (n = 32)	2.12 ± 0.34	3.51 ± 0.49	5.67 ± 0.38	7.51 ± 0.42
p	< 0.05	< 0.05	< 0.05	< 0.05

Footnote: Data are presented as mean ± SD.

Table V. AKP (U/L) in study patients.

Group	Post-treatment, weeks 4	8	12	16
Control group (n = 32)	132.6 ± 13.2	100.3 ± 15.8	83.4 ± 13.4	76.9 ± 13.5
Experimental treatment group (n = 32)	192.5 ± 16.9	153.6 ± 14.8	136.9 ± 14.2	127.3 ± 13.5
p	< 0.05	< 0.05	< 0.05	< 0.05

Footnote: Data are presented as mean ± SD.

contains abundant fibrous proteins which stimulate regeneration of soft tissue and promote early healing of wound surface[22]. Thereby, platelet-rich plasma exerts beneficial effects on lesion repairing in chronic osteomyelitis.

When platelet-rich plasma is combined with bone marrow in an ortholog transplant for chronic osteomyelitis, this leads to the following synergistic effects[23]. First, high concentrations of growth factors in platelet-rich plasma may increase the number of osteoblasts and enhance the growth of capillaries, which further promotes formation of fibroblast matrix, deposition of osteoblasts, and formation of capillaries. Second, platelet-rich plasma can form autologous fibrous protein gel and promote the conduction effect of transplanted bone to provide a microenvironment for proliferation and differentiation of osteoblasts. In turn, bone marrow contains mesenchymal stem cells which have strong self-healing and multi-directional differentiation potentials. In this study, the transplantation was applied with a frequency of 1 time per week and repeated to assure a regular supply of stem cells and growth factors to the lesion. Besides providing autologous bone marrow mesenchymal stem cells, repeated transplantation increases local antibacterial defences.

We observed that at 4, 8, 12 and 16 weeks after this treatment, X-ray and histological scores, and AKP levels were significantly better than in patients on conventional treatment. Increased AKP levels indicate decreased bone damage and increased healing[24,25]. X-ray accurately assesses the healing progress in chronic osteomyelitis[26,27]. We observed an almost complete disappearance of local infected source and strong self-healing capability of autologous bone. Thus, transplantation of platelet-rich plasma combined with bone marrow promotes local blood circulation and shortens the healing time of osteomyelitis cavity. Furthermore, administration of antibiotics directly to the iliac bone further enhances therapeutic effects of this treatment.

Conclusions

The inactivation of infected bone and transplantation of platelet-rich plasma combined with bone marrow exerts significant therapeutic effects in chronic osteomyelitis. This approach removes dead and infected bones, and promotes regeneration of the affected bone.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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