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Case Report

Effect of mesenchymal stromal cell transplantation on congenital pseudarthrosis of the tibia

Ming Liu, Jianli Bu, Yu Yin, Yibing Wang

The Second Department of Orthopaedics, Bethune International Peace Hospital of PLA, Shijiazhuang 050082, P. R. China

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Abstract: This study aimed to investigate the effect of mesenchymal stromal cell (MSC) transplantation on congenital pseudarthrosis of tibia (CPT) treatment. Clinical information of two patients with CPT treated by Bethune International Peace Hospital was analyzed retrospectively, and the effect of MSC transplantation on the treatment of CPT was studied. After bone fixing with a suitable steel plate and local bone grafting, both periosteum ends were injected with 2 ml of MSCs. Four years later, CPT was replaced in both cases and reoperation was performed using Ilizarov technology. After surgery, distal tibia fractures in both cases healed. Notwithstanding no significant effect on the 2 patients after MSC transplantation at the first time, ideal efficacy was achieved after bone grafting surgery. Therapeutic efficacy of MSC transplantation for CPT treatment remains to be further confirmed.

Keywords: Mesenchymal stromal cell, congenital pseudarthrosis of the tibia, Ilizarov ring external fixation technology

Introduction

Congenital pseudarthrosis of the tibia (CPT) is a special bone disconnection that occurs or initiates at birth [1, 2]. CPT tends to generate tibia anterior angular deformity, pathological fracture, and pseudoarthrosis. It is one of the orthopedic chronic and stubborn diseases, and post-operative fracture is a common complication [3-5]. In research conducted by the European Pediatric Orthopaedic Society (EPOS), 340 CPT patients had taken 1287 operations, i.e. an average of 3.79 operations were performed in each patient, indicating that CPT is difficult to cure [6]. There are 23 methods that could be used to treat CPT according to Boyd, and Ilizarov external fixator, intramedullary nail fixation, bilateral bone plate (or metal) fixed bone graft, and free vascularized fibular graft are documented to be the most widely used methods [7, 8]. Unfortunately, the effect is still not satisfactory.

Mesenchymal stromal cells (MSC) are the most mature cells in bone tissue engineering and regeneration at present [9, 10]. In the year 2012, Granchi et al. argued that bone marrow MSC transplantation could induce bone tissue regeneration and promote bone tissue reparation at the broken end of pseudo-joint in patients with CPT [11]. In their study, 10 patients with CPT were treated by MSC derived from the iliac crest (IC-MSC) and bone consolidation was obtained in three patients [11]. Similarly, other scholars also found that transplantation of MSC may be a promising strategy for the treatment of CPT [12-14]. To date, few reports on this field are reported. In this study, the clinical information of two patients with CPT admitted to our hospital was analyzed retrospectively with the intent to investigate the effect of MSC transplantation on CPT.

Case presentation

Two CPT patients who were treated in Bethune International Peace Hospital were enrolled in this study. Complete pre-operative and post-operative imaging data, medical records, pathology of each operation, and other information were collected. The current study was approved by the Medical Ethics Committee of

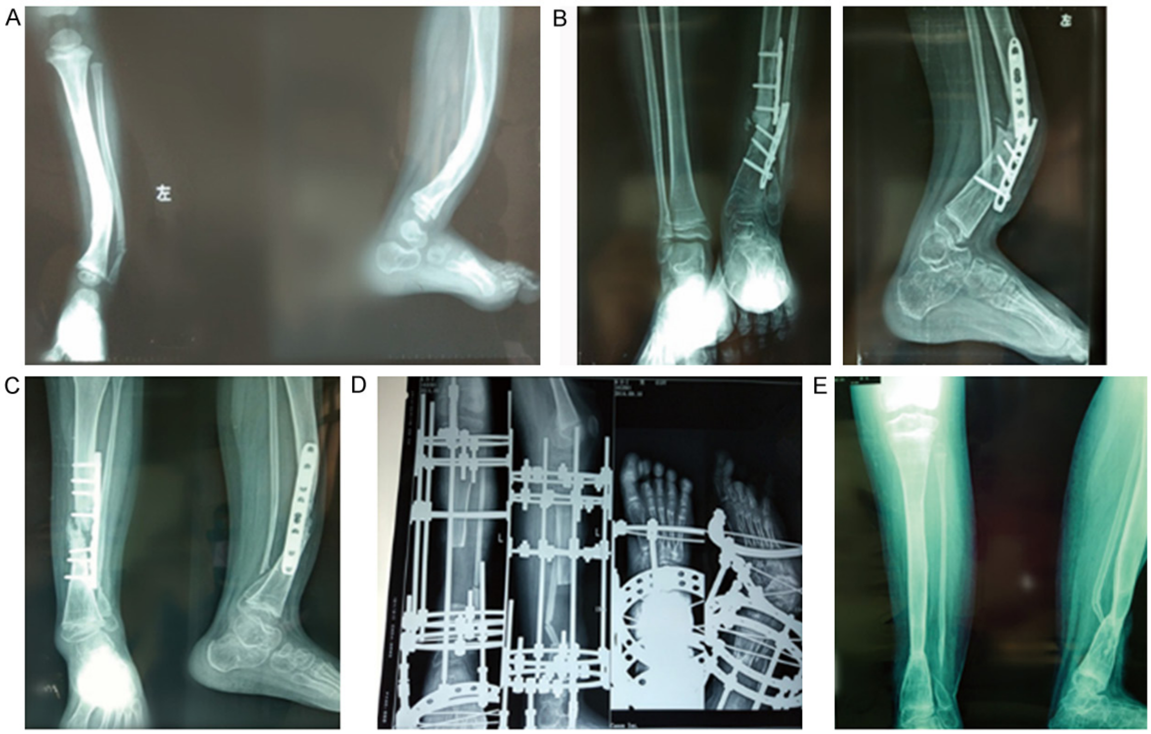

Figure 1. X-ray inspection plates of case 1. Pre-operative X-ray plates of the first surgery on Mar 28, 2007 (A) indicated that the patient was with CPT. Post-operation X-ray inspection after the first operation on Jul 24, 2011 (B) illustrated that the surgery was successful. Pre-operation X-ray of the second operation on Aug 26, 2014 (C) showed that CPT returned again (the lower part of tibia and fibula bone was not continuous). Post-operative X-ray images on Sep 16, 2014 (D) argued that the surgery was successful and X-ray inspection on Apr 16, 2016 (E) demonstrated that the distal tibia fractures basically healed.

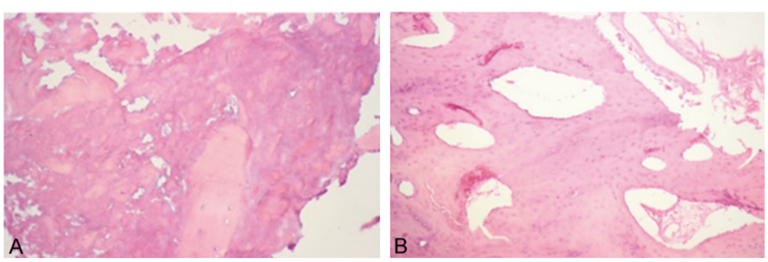

Figure 2. Pathological results (HE staining) of case 1 was similar during the in first surgery (A) and second operation (B). Malnutrition was associated with cystic degeneration, bone degeneration, osteonecrosis, and fibrosis.

Bethune International Peace Hospital of PLA. Informed consents were obtained from the parents of the patients.

Case 1 was a 13-year-old boy firstly visited our hospital when he was 7 years old. Left leg forward bending deformity was found when he was 6 months, and he had taken three surgeries in our hospital. Pre-operative X-ray plates are shown in **Figure 1A**. During the operation, after 20 cm anterior tibia incision was made, peripheral fibrous tissue hyperplasia, bone sclerosis, and periosteal thickening with ring-shaped around pseudarthrosis were found. All the lesions around pseudarthrosis were removed and the medullary cavity tissues were applied for pathological examination. The results show that malnutrition is associated with cystic degeneration, bone degeneration, osteonecrosis, and fibrosis (**Figure 2A**). After the bone was fixed with a suitable steel plate and local bone grafting, both periosteum ends were injected with 2 ml bone marrow MSC. As illustrated in **Figure 1B**, post-operative X-ray inspection shows that the fracture ends were well positioned, the internal fixations were strong, and the tibia pseudarthrosis was well corrected. Unfortunately, CPT reappeared 4 years later. The X-ray inspection (**Figure 1C**) indicated that the lower part of tibia and fibula bone was not

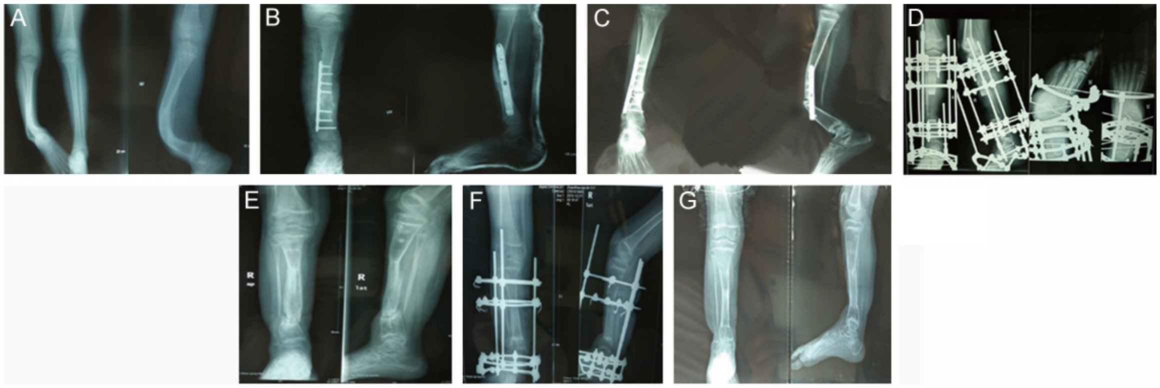

Figure 3. X-ray inspection plates of case 2. Pre-operative X-ray plates of the first surgery on Jun 10, 2010 (A) indicated that the patient was with CPT. (B) Post-operation X-ray inspection after the first operation on Jul 23, 2010 illustrated that the surgery was successful. Pre-operative X-ray of the second operation on Aug 26, 2014 showed that CPT replaced (C) and Ilizarov ring external fixation technique was performed (D, Sep 16, 2014). Post-operative X-ray images 10 months after operation (Jul 12, 2015) demonstrated that the end of the fracture had not been healed (E), and the Ilizarov ring external fixation technique was successfully performed (F, Dec 21, 2015). Follow-up on Dec 26, 2016 showed that the fracture had been healed (G).

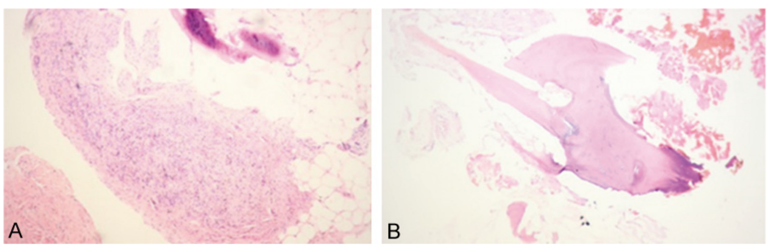

Figure 4. Pathological results (HE staining) of case 2 during the in first surgery (A) and second operation (B) was similar to that of case 1. Periosteum showed chronic inflammation, with fibrous tissue thickened and disorganized. Hyaline degeneration reduced blood vessels, and narrowing even occlusion of the lumen was noted.

continuous. Bone density of fracture ends was increased, the fracture ends bent into an angle, and steel plate breakage was revealed. Reoperation was then performed using Ilizarov technology. Intraoperative exploration found that the soft tissue at fracture was black, the periosteal at fracture ends was thickened, hardened, and absorbed, and medullary cavities were closed. The thickened periosteum and hyperplastic fibrous connective tissue, were hardened and bloodless tibia fracture ends were removed during the operation. Pathological culturing results were similar to that of the first operation (**Figure 2B**). Post-operative X-ray images are presented in **Figure 1D**. Ten months after surgery, distal tibia fractures were healed and the ring external fixator of left lower limb was removed (**Figure 1E**).

Case 2 was a 12-year-old boy first visited our hospital when he was 6 years old. He was found with the right leg forward bending deformity at birth and had undergone four surgeries to date. Pre-operative X-ray images are shown in **Figure 3A**. The first surgical treatment was similar to that of case 1 except that he had ta0 ken umbilical cord blood MSC transplantation. Post-operative X-ray inspection indicated that the operation was successful (**Figure 3B**). Similarly like case 1, CPT reappeared and X-ray performance was consistent with that of case 1 (**Figure 3C**). A therapeutic regimen of Ilizarov technology was taken. Intraoperative exploration and pathological examination results were similar to case 1 (**Figures 3D, 4A**). Post-operative X-ray images (**Figure 3E**) show that the fractures had not healed, thus fracture site osteotomy was applied and 5 mm periosteum was resected, then another Ilizarov was utilized again (**Figure 3F**). Finally, the fracture was healed (**Figure 3G**) and a bone extensive surgery was prepared.

Discussions

CPT is closely related to neurofibroma in soft and bone tissues, and about 50% of children patients suffer from neurofibromatosis at the same time [15]. Thus, CPT is divided into neurofibromatosis type and nonunion neurofibromatosis. The European Pediatric Orthopaedic

Table 1. Review of studies about mesenchymal stromal cell transplantation on treatment of congenital pseudarthrosis of the tibia

Materials	Researchers
MSC derived from the iliac crest, platelet-rich fibrin, and lyophilized bone.	Granchi D et al. [9]
MSC were derived from spare femoral bone marrow.	Ciapetti G et al. [17]
MSC were derived from bone marrow.	Hernigou P [20]
Percutaneous autologous bone marrow transplantation.	Garg NK [21]
Transplantation of MSCs and platelet-rich plasma.	Kitoh H [22]

Surgery (EPOS) put forward the new name of bone fibromatosis for this disease [6], saying that CPT might be the expression of neurofibromatosis in bone tissue. If sufficiently expressed in the bone tissue, it will show clinical manifestations of neurofibromatosis, otherwise it will be called nonunion neurofibromatosis. However, this view needs to be further studied. CPT patients with neurofibromatosis have scattered coffee spots, and their parents, as well as grandparents may also have the same characteristics. In this study, case 1 was called nonunion neurofibromatosis while case 2 was called neurofibromatosis. Scattered coffee spots and multiple nodules on case 2 and his mother were found. Other assumptions [7], such as regional vascular disorder, intrauterine loss, birth fracture, systemic metabolic disorder, and vascular abnormalities were not noted.

To date, CPT treatment is still a challenge and the poor curative effect can be attributed to the following two reasons. First, the cause is still not clear. Second, each treatment protocol has certain defects. No special single method could solve the problems of atrophy of cortical bone, deformity of the joint, and the easily recurrent fracture [16]. For the second surgery, the two cases in the study were subjected to Ilizarov ring external fixation technology which has three merits. First, this technology was cross fixed by application of Kirschner wire diameter 1.5-2.5 mm, and three-dimensional elastic fixation was formed by external ring external fixing frame, making fixation stable and reliable [17, 18]. Second, 0.25 mm was compressed weekly after the operation, which could not oppress the bone end to cause osteonecrosis, prevent bone resorption, and produce stress stimulation to accelerate fracture healing [19]. Finally, as for bone extending, a minor multi-operation method was used, with extension of 1 mm per day and divided into four times [20]. As a result, the bone and soft tissue could grow synchronously under the low tensile force, and the bone tissue could be repaired quickly without the soft tissue damaged.

MSC in bone marrow could differentiate into different connective tissues like bone, fat, cartilage, and muscle [21-23]. Researchers have paid more attention to MSC as it is a suitable source of osteogenic precursors for improving bone regeneration and reparation. MSC could be obtained from the iliac bone marrow in each patient, and it has been an auxiliary for orthopedic surgeons in bone graft [24, 25]. Currently, CPT patients are also treated with MSC [26-28]. However, microenvironment has a potential influence on MSC differentiation. The cases reported to date are reviewed in **Table 1**. Granchi and Devescovi [11] found that only three CPT patients out of 10 patients were healed at the first time. The results of MSC mineralization *in vitro* indicated that MSC could induce bone regeneration and promote bone tissue reparation of pseudarthrosis ends in CPT patients, thus, MSC transplantation might be a useful method for CPT treating. In this study, MSC transplantation was taken for the first operation in both cases, where case 1 was transplanted with bone marrow MSCs and case 2 with umbilical cord blood MSCs. Unfortunately, it was ineffective, and moreover, the pathological results of reoperation were almost the same as initially. Hence, application of MSC transplantation for treating CPT remains to be confirmed further. Pathological results indicated that there was bone malnutrition associated with cystic degeneration, bone degeneration, osteonecrosis, and visible fibrosis generation. The periosteum showed chronic inflammation, fibrous tissue thickening, and a disorderly arrangement, with significant hyaline degeneration, vascular reduction, and stenosis or even occlusion. After bone grafting without MSC transplantation, ideal efficacy was achieved. Pathological results showed that the patients fracture's were healing.

The following experience can be gained from the two cases. First, lesions like thickened periosteum, hyperplasia fibrous tissue, and hardened tibia fracture end without blood should be removed completely. Otherwise, the remaining periosteum and sclerotin can cause disease recurrence. Additionally, diseased fibrous tissue has a strong tendency to reform after bone transplant surgery, especially for young children [29]. Second, the treatment effect of neurofibromatosis is worse than nonunion neurofibromatosis, thus, the healing of case 1 was faster and better than case 2. Therefore, it is still difficult to treat CPT although there are many methods developed. Information about the two CPT children treating and its effect will be reported further so as to provide more information about the treatment of CPT.

Disclosure of conflict of interest

None.

Address correspondence to: Jianli Bu, The Second Department of Orthopaedics, Bethune International Peace Hospital of PLA, No 398, West Zhongshan Road, Shijiazhuang 050082, P. R. China. Tel: +86-0311-87978372; Fax: +86-0311-87978372; E-mail: doctorBJL@sina.com

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