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Case Report Open Access

Umbilical-cord-derived Mesenchymal Stem Cell Transplantation Ameliorated Severe Leg Ulcers in a Patient with Rheumatoid Arthritis: A Case Report and Review of the Literature

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Abstract

Most lower extremity ulcers in patients with rheumatoid arthritis (RA) will become chronic non-healing ulcers without treatments. However, established therapies, including glucocorticoid and immunosuppressive agents didn't show a good efficacy. Some studies have shown umbilical-cord-derived mesenchymal stem cells (UC-MSCs) could accelerate the healing of cutaneous wounds in mice. But no clinical trials of UC-MSCs in cutaneous wound healing have been reported in human. Here we found that UC-MSCs transplantation promoted the healing of ulcers in a RA patient who underwent no improvement with established therapies. This case showed the prospective of UC-MSCs' application in treating recurrent ulcers in RA patients.

Keywords: Mesenchymal stem cell; Stem cell; Transplantation; Ulcers; Rheumatoid arthritis

Introduction

Lower extremity ulcers are recognized complications of rheumatoid arthritis (RA), and most of them will become chronic non-healing ulcers without treatments. Established therapies, including glucocorticoid and immunosuppressive agents can take a long time to heal the wounds and, additionally, not all patients respond well to these treatments [1]. In recent years, endothelial progenitor cells (EPCs) [2], bone-marrow-derived mononuclear cells (BM-MNCs) [3], fibrocytes [4] and karotinocytes [5] have been shown to be helpful in the healing process of leg ulcers. In a rat diabetic wound healing model, systemic and local treatment with bone-marrow-derived mesenchymal stem cells (BM-MSCs) also demonstrated efficacy [6].

Umbilical-cord-derived mesenchymal stem cells (UC-MSCs) are stem cells derived from the umbilical cord stroma, which can differentiate into a number of cell types including adipocytes, chondrocytes, osteocytes, cardiomyocytes, skeletal myocytes, hepatocytes, insulin-producing cells, as well as neuron-like cells [7]. They have been shown to accelerate the healing of cutaneous wounds in mice [8,9]. However, no clinical trials of UC-MSCs in cutaneous wound healing have been reported in RA patients. Here we present the first case of a 42-year-old RA patient with severe large ulcers on the leg who was treated successfully by UC-MSCs transplantation.

Material and Methods

Case presentation

A 42-year-old female with 28-year history of RA (fulfilling the ACR classification criteria [10]) presented with recurrent ulcers on her right leg for 6 months. The ulcers distributed along the right thigh with a size of 40 cm×50 cm, and right gluteal, extensor aspect of right knee and lateral aspect of right ankle with the sizes from 2 cm×3 cm to 7 cm×8 cm (Figures 1a and 1b). There were yellow purulent secretion, scattered necrotic tissue and hyperalgesia at the ulcers. Chest CT showed lung interstitial disease combined with infection. After therapy with anti-infection, immunosuppression, and regional clearances at local hospital for months, her condition did not improve. She was then transferred to our hospital for further evaluation and therapy. Review of systems revealed 28 years of repeated symmetric swelling and tenderness of small joints, mainly involving two hands. Her past medical history was negative aside from hypertension (HP) and type 2 diabetes mellitus (type 2 DM).

On examination, the patient was conscious and afebrile. Her lungs were clear to auscultation except for crepitation on the left lower lung. Both hands showed ulnar drift deformity, with limited extension of the wrists. No swelling or tenderness of joint was found, with disease activity score (DAS28)<2.6 [11].

C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were 90 mg/dl and 43 mm/h respectively (normal range: CRP 0-8 mg/L, ESR 0-20 mm/h). Fasting blood glucose was 4.7 mmol/l (normal range: 3.9-6.1 mmol/L). Skull CT and MRI showed no significant abnormalities. Secretion culture of the ulcers revealed *Staphylococcus aureus* and *Pseudomonas aeruginosa* infection.

During hospitalization, the patient got developing headache, but she refused lumbar puncture. She also denied surgical intervention and was then treated with glucocorticoid, cyclophosphamide (I.V.), broad spectrum antibiotics, debridement and necessary nutritional support. BP and blood glucose were controlled stably. However, two weeks later the ulceration still had not ameliorated. So allogeneic UC-MSCs transplantation was then considered.

The transplantation was administered after the approval of The Ethics Committee of the Affiliated Drum Tower Hospital of Nanjing University Medical School and patient's informed consent. UC-MSCs were obtained from Jiangsu Stem Cell Center, Jiangsu, China, and

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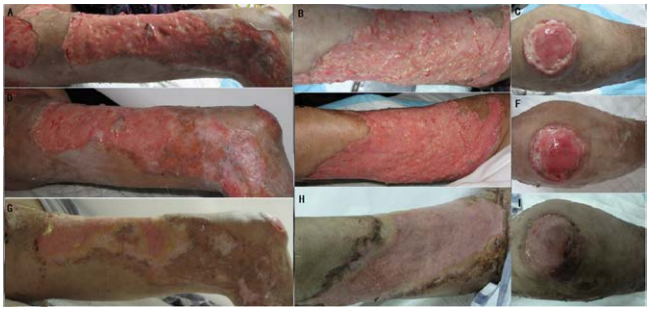

Figure 1: Healing of the ulcers on the right thigh (outer side and inner side) and knee after UC-MSCT. (A-C) before MSCT; (D-F) about one month after the first MSCT; (G-I) about three months after the first MSCT.

prepared for transplantation as previously described [12]. Briefly, umbilical cord (UC) was obtained from local maternity hospitals after normal deliveries and then digested and cultured for 2 generations. After 2 passages cells were harvested. Flow cytometric analysis showed CD29, CD44, and CD105 expression of >95%, in parallel with CD45, CD34, CD14, and HLA-DR expression of <2%. We used good manufacturing practice (GMP) conditions and clinical-grade reagents for preparation of the cells.

A total of 5×10^7 cells (1×10^6 cells per kg of the patient's weight) were administered intravenously within 20 minutes after intravenous infusion of methylprednisolone at 40 mg/day × one time before the transplantation to avoid allergy. The procedure for UC-MSC transplantation was the same as our previous usage in SLE patients [13]. The patient was carefully monitored during and 24 h after the treatment (including heart rate, blood pressure and consciousness) and no obvious side effects were observed.

Results

Ulcers healing

About one month after the first transplantation, we could see growth of granulation tissue at the ulcers (Figure 1D-1F). Two additional transplantations with the same amount of MSCs 1.5 months and 3 months after the first transplantation were given. And we could see significant improvements of the ulcers (Figure 1). After about 3 months the ulcers had significantly healed, with methylprednisolone 10 mg/day for maintenance (Figures 1G-1I).

Infection

However, the patient's headache continued developing, accompanied by fever and vomiting. Cerebrospinal fluid (CSF) examination demonstrated 43 leukocytes/μl (normal range: 0-8/μL) (58% neutrophils, 38% lymphocytes and 4% monocytes) under microscope with clear appearance, which indicated central nervous system (CNS) infection. CSF biochemistry showed 1.37 mmol/l glucose (normal range: 2.5-4.5 mmol/L), 125.7 mmol/l chloride (normal range: 120-132 mmol/L), 908.6 mg/l protein (normal range: 150-450 mg/L). Pathologic diagnosis indicated 12/μl *Cryptococcus* with prolification and mild CNS infection. CSF culture indicated *Cryptococcus neoformans* proliferation, which was also present in blood culture. Sputum culture and fungi culture found yeast-like *fungi*, clindamycin-resistant *Staphylococcus aureus*, methicillin resistant *staphylococcus aureus*(+) and *Candida albicans*. After 2 weeks of antibiotic and anti-fungi therapy, with the use of vancomycin, ciprofloxacin, 5-fluorouracil, fluconazole intravenously, her headache alleviated and the protein level of CSF decreased. However, her headache aggravated and both *Cryptococcus* and protein level of CSF increased after another 11 days. And we began to treat her with additional intrathecal injections of amphotericin B from then on. In spite of the effectiveness of amphotericin B, her liver and renal function gradually worsened because of the side effect. After injections for 3 months, we had to stop amphotericin B. And a few days later, she succumbed to the CNS infection finally.

Discussion

A postal survey administered to 1,130 RA patients in West Yorkshire, England, taken in 2008, revealed a point prevalence of foot ulceration in RA of 3.39%, and an overall prevalence of 9.73% [14]. In a 3 year study of 366 RA patients, evaluated at Georgetown Division of Rheumatology, 4.37% had active leg ulcers [8]. Leg ulcers in RA patients tended to be resistant to local therapy; conventional treatments often aimed at stabilization of the underlying autoimmune disease, e.g. with high doses of glucocorticoids or other potent immunosuppressive agents. In the Georgetown study, only 31.25% had healed with the treatment of non-biologic disease-modifying anti-rheumatic drugs (DMARDs) alone or combined with anti-TNFα agents after a mean of 22.76 months of follow-up with ameanhealing time of 32.7 months [1]. Some studies have shown the efficacy of autologous bone marrow derived stem cell transplantation in chronic cutaneous wounds, such as end-stage chronic critical limb ischemia or diabetic foot [15,16]. The umbilical-cord-blood-derived MSCs (UCB-MSCs) when applied to cutaneous wounds, demonstrated enhancement in wound healing in an immunodeficientBalb/C SCID mouse model [8]. Tark et al. applied UCB-MSCs to diabetic wounds in a study utilizing Leprdb mouse model, demonstrating that MSCs from human cord blood accelerated healing when injected locally [9]. However, no clinical trials of umbilical-cord-derived MSCs in cutaneous wound healing were reported despite the encouraging efficacy in animal models.

In our case, the patient had a long duration of RA, with concomitant diabetes and infection. After sufficient anti-infection and debridement, no trend of improvement could be seen. While after transplantation of UC-MSCs, the sizes of ulcers began to decrease in 2 months, which indicated the prospective of UC-MSCs transplantation in recalcitrant ulcer treatment.

Some previous researches demonstrated that stem cells could mobilize and home to ischemic and wounded tissue where they secreted chemokines and growth factors that promoted angiogenesis and extracellular matrix (ECM) remodeling, creating a local environment conducive to wound healing [17-20]. Besides multiple-differentiating capacity in regeneration, UC-MSCs seem to have a profound effect on immune-regulation [21,22]. In patients with autoimmune diseases, proinflammatory environment may be an important reason for non-healing ulcers. So the inhibition of inflammatory factors by UC-MSCs could play a crucial role in the underlying mechanism.

This patient succumbed to CNS infection which seemed to exist before transplantation. Impaired immunity due to long term use of glucocorticoid and immunosuppressive and exposure to infection because of large ulcers should be responsible for her death. Though her condition improved with the intrathecal injections of amphotericin B, the side effect of liver and renal function worsening forced us to stop it, which led to her final death from the development of CNS infection. Additionally, a systemic review and meta-analysis of clinical trials did not find an association of MSCT with infection, organ system complications or death [23]. Our own experience of MSCT in systemic lupus erythematosus also showed long-term safety [13]. Several studies

even found infusion of MSC could reduce the risk of infection and improve survival in human [24] or mice [25]. So we don't think the infection and death of the patient were due to transplantation.

The potential risks of MSC therapy, including (i) immunogenicity of the cells, (ii) biosafety of medium components, (iii) risk of ectopic tissue formation, and (iv) potential *in vitro* transformation of the cells during expansion, have been taken into account in one review [22]. According to currently available experimental and clinical data, MSC treatment for autoimmune disorders is feasible and safe [13,22,26]. Nevertheless, to demonstrate the exact mechanism, the safety, treatment efficacy as well as the optimal dose and interval, more clinical controlled trials of UC-MSCT with a long-term follow up in human subjects are necessary.

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