Synergistic Effect of Bone Marrow-Derived Mesenchymal Stem Cells and Platelet-Rich Plasma in Streptozotocin-Induced Diabetic Rats

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Background: Diabetic wounds are a major clinical challenge, because minor skin wounds can lead to chronic, unhealed ulcers and ultimately result in infection, gangrene, or even amputation. Studies on bone marrow derived mesenchymal stem cells (BMSCs) and a series of growth factors have revealed their many benefits for wound healing and regeneration. Platelet-rich plasma (PRP) may improve the environment for BMSC development and differentiation. However, whether combined use of BMSCs and PRP may be more effective for accelerating diabetic ulcer healing remains unclear. Objective: We investigated the efficacy of BMSCs and PRP for the repair of refractory wound healing in a diabetic rat model. Methods: Forty-eight rats with diabetes mellitus induced by streptozotocin were divided into four groups: treatment with BMSCs plus PRP, BMSCs alone, PRP alone, phosphate buffered saline. The rate of wound closure was quantified. A histopathological study was conducted regarding wound depth and the skin edge at 7, 14, and 28 days after surgery. Results: Wound healing rates were significantly higher in the BMSC plus PRP group than in the other groups. The immunohistochemistry results showed that the expression of platelet/endothelial cell adhesion molecule 1, proliferating cell nuclear antigen, and transforming growth factor-β1 increased significantly in the BMSC plus PRP group compared to the other treatment groups. On day 7, CD68 expression increased significantly in the wounds of the BMSC plus PRP group, but decreased markedly at day 14 compared to the controls. Conclusion: The combination of BMSCs and PRP aids diabetic wound repair and regeneration. (Ann Dermatol 26(1) 1~10, 2014)

Keywords: Bone marrow-derived mesenchymal stem cell, Diabetes mellitus, Platelet-rich plasma, Wounds

INTRODUCTION

Diabetic foot ulcers (DFUs), a significant complication in diabetes, have a significant impact on the quality of life and mortality of patients1. It is estimated that approximately 170 million patients have diabetes worldwide2. Normal wound repair is a complex and dynamic process, including inflammation, angiogenesis, granulation tissue formation, and remodeling3. Diabetic wounds exhibit impaired angiogenesis, reduced growth factor levels, and reduced chemotactic ability to recruit inflammatory cells to the wound4. Poor vascularization and maintenance of a chronic inflammatory state limit the healing capacity of DFUs. Many studies have suggested that bone marrow derived mesenchymal stem cells (BMSCs) contribute to rapid healing in a variety of reconstructive and restorative surgeries5,7. In addition, platelet-rich plasma (PRP) therapy is an efficacious treatment modality for DFUs6,9. BMSCs represent a small portion of the cells in the bone
marrow stromal compartment but have the potential to differentiate into multiple lineages, including osteoblasts, adipocytes, myoblasts, epithelial, and neurons cells. In diabetics, the contribution of BMSCs to skin repair may rely on independent immunomodulatory actions and recruitment of cells to neo-angiogenic sites to accelerate revascularization. Neovascularization supports newly formed tissue and transports circulating cells to the wound region. BMSCs recruit endogenous stem cells to neo-angiogenic sites to accelerate the revascularization process and participate in the inflammatory phase of wound repair to initiate wound healing. BMSCs can be obtained in a culture, making them a cost-effective alternative for the treatment of chronic wounds.

PRP has been used in a wide variety of clinical treatments and surgical procedures, specifically in the field of soft tissue ulcers and chronic skin diseases. PRP contains more than 20 types of growth factors, such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and transforming growth factor (TGF)-β. These factors not only regulate cell migration and proliferation but also remodel the extracellular matrix (ECM) and promote angiogenesis, creating a beneficial environment that enhances wound healing. In the present study, we evaluated the beneficial effects of BMSCs and PRP for diabetic wound repair.

**MATERIALS AND METHODS**

**Animals**

Forty-eight male Sprague-Dawley rats (weight, 200 to 220 g) were obtained from the Animal Center of Wenzhou Medical College (Wenzhou, China). The animals were housed in spacious cages and were given food and water ad libitum. The animal room was ventilated and was under a 12 hours/12 hours light-dark schedule. All animal care complied with the legal guidelines, and the experimental procedures were conducted following approval by the Wenzhou Medical College Animal Policy and Welfare Committee (Approval Document NO. 2009/APWCWC/0031).

**Streptozotocin-induced diabetes**

Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) (Sigma, St. Louis, MO, USA) dissolved in sodium citrate (0.1 mM, pH 4.5) at a dose of 60 mg/kg as described previously. Blood glucose levels were measured by blood tail sampling with a glucometer (B. Braun, Seoul, Korea). Rats with glucose levels > 300 mg/dl (16.7 mmol/L) were considered diabetic and manifested the diabetic state for 4 weeks prior to the study.

**Bone marrow derived mesenchymal stem cell isolation and culture**

BMSCs were harvested from the femurs and tibias of normal male rats (100 g) under aseptic conditions according to methods described previously with minor modifications. Briefly, the cells were cultured in DMEM/F-12 medium (Gibco, Grand Island, NY, USA), supplemented with 10% fetal bovine serum (Gibco) 100 U/ml penicillin (Invitrogen, Carlsbad, CA, USA), and 100 μg/ml streptomycin (Invitrogen). BMSCs at passage 3 were used for treatment.

BMSCs harvested at passage 2 were assessed by flow cytometry (Epics-XL; Beckman Coulter, Tokyo, Japan). The cells were incubated with fluorescein isothiocyanate-conjugated polyclonal antibodies against rat CD29, CD45, CD90, and CD11b/c (all from Biolegend, San Diego, CA, USA).

**Preparation of platelet-rich plasma**

PRP was harvested via the double centrifugation of blood, as described previously. Human blood was collected from healthy volunteers in a container containing citric acid-citrate-dextrose anticoagulant. The whole blood was centrifuged at 2,400 rpm for 10 minutes at 22°C. The sample was further centrifuged at 3,500 rpm for 15 minutes to obtain the platelet concentrate. Platelets were counted using an automated hematology analyzer (XE-2100; Sysmex, Kobe, Japan). Platelet counts > 1×10⁹ platelets/ml were used. A solution of 1,000 IU thrombin (Sigma) and 10% calcium chloride was added to the PRP at a volume ratio of 10 : 1 to activate the platelets. The sample was incubated for 1 hour at room temperature, centrifuged at 3,000 ×g for 20 minutes, and the supernatant was collected. The supernatant (activated PRP) was stored at −80°C until use.

**Wound model and treatments**

Rats with diabetes mellitus induced by STZ (DMIS rats) were weighed and anesthetized with 60 mg/kg Nembutal (Sigma). Abdominal hair was clipped and depilated, and the skin was cleansed with Betadine and 70% alcohol solution. A standardized 2 cm² full-thickness wound was made. Each rat had two wounds created, one on the left side and one on the right side. The wounds were photographed and covered with semi-occlusive polyurethane dressings (Tegaderm; 3M Health Care, St. Paul, MN, USA) to avoid desiccation. Approximately 6×10⁶ BMSCs were resuspended in 0.2 ml phosphate buffered saline (PBS) and injected at 10 different sites. PRP was injected through the dressing into the wounds. All rats were