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ORIGINAL ARTICLE

Transplantation of bone marrow-derived mesenchymal stem cells facilitates epithelial repair and relieves the impairment of gastrointestinal function in a rat model of enteritis

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Summary
Background: To examine whether the bone marrow-derived MSCs (BM-MSCs) could facilitate epithelial repair and thereby reduce impairment of gastrointestinal structure and function in chronic murine enteritis induced by indomethacin (IDM).
Methods: MSCs were isolated from young Sprague-Dawley rats. After *in vitro* expansion and characterization, BM-MSCs were labelled with the fluorescent dye PKH26 and transfused, via the tail veins, into rats with enteritis induced by IDM. The controls were infused with sterile saline. The homing and differentiation of the transplanted BM-MSCs were tracked by means of fluorescent staining. The clinical symptoms of the IDM-treated rats were assessed, and the macroscopic and microscopic histological evaluations of the intestines were performed.
Results: Compared to controls that received saline infusion, BM-MSCs treated rats showed lower scores of weight loss, stool consistency, and stool blood. The PKH26-labelled cells resided at the injured intestine, where they co-localize with the proliferating cell nuclear antigen (PCNA), Lgr-5, and Msi-1. The BM-MSCs treated rats showed significantly higher intestinal villi with larger areas relative to the saline-treated rats.

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Conclusion: The transplanted BM-MSCs are able to recognize the injured intestine, where they proliferate and transdifferentiate into intestinal stem cells which repair the injured intestinal tissues. Therefore, BM-MSCs are able to relieve the impairment of gastrointestinal function in IMD-treated rats.

Introduction

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are complex gastrointestinal disorders characterized by chronic, local, and systemic inflammation and a spontaneously relapsing course. In patients with IBD, mucosal ulceration and inflammation may cause abdominal pain, chronic diarrhea, anemia and malnutrition. Although the aetiology of IBD remains unclear, it is generally considered that autoimmunity plays a central role in the pathogenic interplay between genetic predisposition, microbial infection and environmental insults [1]. In a susceptible host, inappropriate and prolonged immunological response to mucosal flora results from failure of regulatory T cells to restrict the activity of self-reactive effector T cells [2—4].

Although immunosuppression with drugs such as corticosteroids, azathioprine, cyclosporine and tacrolimus form the mainstays of therapy in IBD, observations of patients undergoing bone marrow transplantation for other diseases experienced improvement and sometimes complete remission of their CD has culminated in exciting programs offering haematopoietic stem cell transplantation (HSCT) as a potential for cure in IBD [5]. For example, the CD was improved in a patient who underwent allogeneic bone marrow transplant for lymphoma [6]. In another report, patients showed no clinical or laboratory evidence of CD recurrence in the next seven years after an autologous HSCT for non-Hodgkin's lymphoma [7]. In a recent phase I-II study, all four CD patients had active disease at the time of transplantation after immunosuppressant treatment and anti-tumor necrosis factor (TNF) therapy. Three months after transplant, all patients had achieved clinical remission, and complete endoscopic remission was achieved in two patients out of three [8].

In addition to haematopoietic stem cells (HSC), mesenchymal stem cells (MSCs) are other candidates for treatment of IBD [9]. MSCs may be isolated from bone marrow, skeletal muscle, adipose tissue, synovial membranes and other connective tissues of human adults as well as cord blood and placenta. Depending upon the environment, MSCs give rise to many cell lineages including epithelial cells, astrocytes, osteoblasts, chondrocytes, adipocytes and muscle, promoting regeneration of damaged tissue *in vivo* [10]. In preclinical models, MSCs differentiate into cells that can reduce the effects of inflammatory disease. They were shown to down-regulate immune responses through intrinsic properties, such as production of IL-10. They also promoted epithelial cell repair in the gastrointestinal tract [11]. In recent animal studies, MSCs were infused either intravenously or intraperitoneally and improved experimental colitis [12—14]. But, the results reported in a few human studies with IBD patients have been inconsistent [15], suggesting further studies are still necessary.

In the present study, we examined whether the bone marrow-derived MSCs (BM-MSCs) could facilitate epithelial repair and thereby reduce impairment of gastrointestinal structure and function in chronic murine enteritis induced by indomethacin (IDM). IDM-induced enteritis is one of established animal model of IBD [16] and has been used to test the therapeutic efficacy of various drugs [17—19]. Based on the beneficial effects of BM-MSCs seen in the other animal models of IBD as mentioned above [12—14], we hypothesized that BM-MSCs would facilitate epithelial repair and thereby reduce impairment of gastrointestinal structure and function in IDM-treated animals.

Material and methods

Animals

Male Sprague-Dawley (S-D) rats, two-month old, body weight 280—300 g, were purchased from the animal facility of Second Affiliated Hospital of Harbin Medical University, China. Animals were maintained in an environmentally controlled room (20—21 °C, 60% humidity, 12:12-h light-dark cycle) with sterile water and standard laboratory rat chow *ad libitum*. Totally, 28 rats were enrolled in the study. And four rats were assigned into donor group for bone marrow mesenchymal stem cells harvest, the other 24 rats assigned to recipient group ($n = 24$) were divided into two groups randomly (IDM + MSCs $n = 12$, IDM + saline $n = 12$). The experimental protocol was approved by the Harbin Medical University Animal Care and Use Committee. All animals were cared in accordance with National Institutes of Health guidelines for the use of experimental animals.

Isolation and expansion of BM-MSCs

The approach of isolation and expansion of BM-MSCs was followed by our previous study [20]. In detail, two-month old S-D rats ($n = 4$) were killed by cervical dislocation. The bilateral femurs were aseptically excised after removing connective tissues around the bones. Then, femurs were stored in phosphate-buffered saline (PBS) supplemented with 1 × penicillin/streptomycin on ice and the surface of them was further trimmed and cleaned. The bones were broken and the bone marrow was flushed out of the medullar cavity using L-DMEM (Dulbecco's Modified Eagle Medium (D-MEM) (1X), liquid, no glucose, GIBCO®). The dispersed bone marrow cells were centrifuged for 5 min at 350 × *g*. After removal of the supernatant, cells were plated in plastic tissue culture flask (Corning, NY, USA) with L-DMEM supplemented with 10% fetal bovine serum (FBS; Shanghai Solarbio)