Cytotherapy, 2017; 19: 1208–1224

International Society for Cellular Therapy
ISCT®

Functional recovery upon human dental pulp stem cell transplantation in a diabetic neuropathy rat model

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Abstract

Diabetic neuropathy (DN) is among the most debilitating complications of diabetes. Here, we investigated the effects of human dental pulp stem cell (DPSC) transplantation in Streptozotocin (STZ)-induced neuropathic rats. Six weeks after STZ injection, DPSCs were transplanted through two routes, intravenous (IV) or intramuscular (IM), in single or two repeat doses. Two weeks after transplantation, a significant improvement in hyperalgesia, grip-strength, motor coordination and nerve conduction velocity was observed in comparison with controls. A rapid improvement in neuropathic symptoms was observed for a single dose of DPSC IV; however, repeat dose of DPSC IV did not bring about added improvement. A single dose of DPSC IM showed steady improvement, and further recovery continued upon repeat IM administration. DPSC single dose IV showed greater improvement than DPSC single dose IM, but IM transplantation brought about better improvement in body weight. A marked reduction in tumor necrosis factor (TNF) α and C-reactive protein (CRP) levels was observed in the blood plasma for all treated groups, as compared with controls. With respect to inflammatory cytokines, repeat dose of DPSC IM showed further improvement, suggesting that a repeat dose is required to maintain the improved inflammatory state. Gene expression of inflammatory markers in liver confirmed amelioration in inflammation. Arachidonic acid level was unaffected by IV DPSC transplantation but showed noticeable increase through IM administration of a repeat dose. These results suggest that DPSC transplantation through both routes and dosage was beneficial for the retrieval of neuropathic parameters of DN; transplantation via the IM route with repeat dose was the most effective.

Key Words: *C-reactive protein, dental pulp stem cells, diabetic neuropathy, hyperalgesia, inflammatory cytokines, intramuscular administration, nerve conduction velocity*

Introduction

Diabetic neuropathy (DN) is a disorder of the nervous system and a common early developing complication in both type 1 and type 2 diabetes [1]. More than half of all long-standing diabetic patients eventually develop neuropathy leading to foot ulceration, with a lifetime risk of consequent lower extremity amputations estimated to be up to 15% [2]. Current treatments are largely focused on controlling hyperglycemia and management of neuropathic pain. This includes tight glycemic control and symptomatic treatment by antidepressants, anticonvulsants, opioids and topical agents [3]. Most of these medications have side effects and fail to target the inflammatory milieu that arises in DN [4]. An additional therapy of transplantation of pancreatic islets has been tried, but its use is restricted by a lack of donor organs and recurrent autoimmune response against islets [5–7]. This suggests an urgent need for a new disease-modifying therapeutic approach in DN.

Based on their unique properties of homing and immunomodulation, bone marrow mesenchymal stromal cells (BM-MSCs) [8–10] are considered to be a viable strategy to restore peripheral nerve function [11–16]. MSCs exhibit immense therapeutic potential because they can migrate and home on to injured sites where they not only regenerate tissues but also secrete trophic factors and paracrine mediators, in addition to their ability to differentiate into various

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(Received 7 March 2017; accepted 20 July 2017)

ISSN 1465-3249
http://dx.doi.org/10.1016/j.jcyt.2017.07.009

cell types [17–19]. Also, they interact with dendritic cells, T cells and natural killer (NK) cells to modulate immune responses, by inhibiting tumor necrosis factor (TNF) α and interferon (IFN) γ and by increasing interleukin (IL)-10 [20–25]. Their immune-evasiveness makes BM-MSCs appropriate for both autologous and allogenic transplantation [26].

However, BM as a source has its own limitations. BM aspiration has the risk of sciatic nerve crush [27] and usually it is not considered to be a discarded tissue like teeth extracted from healthy individuals for cosmetic reasons or umbilical cord blood and matrix. Human biological discarded tissues are increasingly gaining importance for their lesser ethical concerns.

Dental pulp is presently considered to be a promising potential source for MSCs, especially for transplantation in neurological disorders, due to its cranial neural crest origin [28] and ease of isolation from extracted adult teeth. Dental pulp stem cells (DPSCs) are multipotent MSCs of neural crest derivation that exhibit a similar mesenchymal marker profile and comparable immunomodulatory and homing properties to BM-MSCs [29,30]. Being ecto-mesenchymal in origin, they are also reported to be providers of neurotrophic factors [31–34].

Transplantation of murine DPSCs has shown improvement of pancreatic damage, renal function and hyperalgesia in a Streptozotocin (STZ)-induced type 1 diabetic mouse model [35]. Recent work on the effect of transplantation of rat DPSCs in a DN rodent model has shown improvement of behavioral parameters along with nerve conduction [36]. However, the mechanism of action of these DPSCs with respect to the modulation of the inflammatory milieu is not clear. Also, clinical parameters of crucial translational significance, such as the optimal route and dosage of stem cell administration, are yet to be assessed for DN. In addition, there is yet no study on the effect of human DPSCs in an *in vivo* DN model. Thus, the goal of this present study was to investigate the effect of human DPSCs on body weight, diabetic, neuropathic and inflammatory parameters, when administered through local (intramuscular [IM]) or systemic (intravenous [IV]) routes, in single and two repeat doses.

Materials and methods

Animals

Male Wistar rats (16 weeks old, weighing 250–300 g) were obtained from Al-Ameen College of Pharmacy and housed 2–3 per cage under controlled laboratory conditions (22°C ± 1°C on a 12-h light/dark cycle with standard laboratory rat chow with water *ad libitum*) and were acclimatized for 1 week prior to the beginning of the study. As described earlier by Minaz and Razdan [37], diabetes was induced by a single intraperitoneal (IP) injection of STZ (Sigma Aldrich; 55 mg/kg body weight in citrate buffer with pH 4.5) and was verified by measuring fasting serum glucose concentration, post 72 h of STZ administration. Rats with fasting blood glucose levels >250 mg/dL were considered diabetic. Subcutaneous administration of 3 IU/d of insulin was performed throughout the period of study to mimic sustainment of long-term diabetes in humans. Age- and weight-matched male Wistar rats were used as experimental controls. All animal procedures were performed in accordance with regulations specified by the Institutional Animal Ethics Committee of Committee for the Purpose of Control And Supervision of Experiments on Animals. Body weight of control and diabetes-induced rats was estimated using a digital balance (Essae DS-252) before and after STZ administration and also every week post–DPSC transplantation (PDT). The difference in body weight in comparison with the weight measured at the beginning of the study were determined and plotted with respect to the duration of the rat in study. Blood was withdrawn through the retro-orbital plexus of overnight-fasted rats, before and after STZ administration, and serum was separated by using a cold centrifuge (Cooling Microfuge CM-12). Fasting serum blood glucose levels were measured by using a standard glucose kit (Agappe Diagnostics LTD.).

Isolation and culture of DPSCs

Normal third molar teeth were collected from 12 healthy patients 18–40 years of age, undergoing surgery for various medical reasons at the Department of Dentistry, Manipal Hospital, Bengaluru, India (approved by the Institutional Ethical Committee, Manipal Hospital, Bangalore). The isolation procedure for each tooth was carried out as per our previous report [38]. In brief, the dental pulp tissue was aseptically removed and digested enzymatically using 2 mg/mL of collagenase (Sigma-Aldrich) for 1 h at 37°C in a humidified chamber. Isolated cells were plated in knockout Dulbecco's modified Eagle's medium (KO-DMEM; GIBCO-BRL) containing 10% fetal bovine serum (FBS), 5 mmol/L L-glutamine and 50 U/mL penicillin–streptomycin (all purchased from Himedia) and incubated at 37°C with 5% CO_2 in a humidified chamber. Further, the cells were sub-cultured and used for transplantation. For basic mesenchymal characterization, DPSCs between passages three and six were harvested with 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA; GIBCO-BRL) and resuspended in wash buffer containing 0.01% sodium azide in ice cold phosphate-buffered saline (PBS), blocked with 2% FBS and incubated at room temperature for 10 min. The