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Transplantation of bone marrow stromal cells for peripheral nerve repair

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Abstract

Cell transplantation using bone marrow stromal cells (BMSCs) to alleviate neurological deficits has recently become the focus of research in regenerative medicine. Evidence suggests that secretion of various growth-promoting substances likely plays an important role in functional recovery against neurological diseases. In an attempt to identify a possible mechanism underlying the regenerative potential of BMSCs, this study investigated the production and possible contribution of neurotrophic factors by transected sciatic nerve defect in a rat model with a 15 mm gap. Cultured BMSCs became morphologically homogeneous with fibroblast-like shape after *ex vivo* expansion. We provided several pieces of evidence for the beneficial effects of implanted fibroblast-like BMSCs on sciatic nerve regeneration. When compared to silicone tube control animals, this treatment led to (i) improved walking behavior as measured by footprint analysis, (ii) reduced loss of gastrocnemius muscle weight and EMG magnitude, and (iii) greater number of regenerating axons within the tube. Cultured fibroblast-like BMSCs constitutively expressed trophic factors and supporting substances, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), ciliary neurotrophic factor (CNTF), collagen, fibronectin, and laminin. The progression of the regenerative process after BMSC implantation was accompanied by elevated expression of neurotrophic factors at both early and later phases. These results taken together, in addition to documented Schwann cell-like differentiation, provide evidence indicating the strong association of neurotrophic factor production and the regenerative potential of implanted BMSCs.

Keywords: Bone marrow stromal cells; Neurotrophic factor; Regeneration; Sciatic nerve; Schwann cells; Transplantation

Introduction

The ability of regenerating neurites to penetrate through the structurally altered extracellular matrix, surrounding tissues, and infiltrating cells at the injured site to reach synaptic targets plays an important role in the regeneration of peripheral nerves. Treatment of peripheral nerve defects is primarily aimed at recreating continuity of the nerve to allow axonal re-growth into the nerve stump distal to the lesion. Therapeutic approaches for the reconstruction of the peripheral nerve defects include end-to-end suturing, fascicular suturing, nerve grafts, and nerve conduits. Evidence indicates that nerve grafting is essential to the reconstruction of long nerve defect. Recently, cell transplantation has become the focus of attention, especially that of Schwann cells, and reliable outcomes have been achieved in the regeneration of the sciatic nerve (Bryan et al., 1996). In addition, the implantation of neural stem cells, bone marrow stromal cells (BMSCs), or fibroblasts has been shown to exert a beneficial effect on peripheral nerve regeneration (Shen et al., 1999; Dezawa et al., 2001; Cuevas et al., 2002, 2004; Murakami et al., 2003; Heine et al., 2004; Mimura et al., 2004). Thus, cell transplantation has been proposed as a method of improving peripheral nerve regeneration.

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Multipotent stem cells, which participate in normal replacement and repair while undergoing self-renewal, have been detected in multiple tissues in adults. Stem cells are being investigated as replacement therapy for a variety of disorders and can be engineered to deliver appropriate support to the intrinsic cells in a diseased organ system (Park et al., 2002; Murakami et al., 2003; Heine et al., 2004). Several investigators have published reports on hematopoietic stem cells and mesenchymal stem cells derived from bone marrow. Bone marrow mesenchymal stem cells, which are also known as bone marrow stromal cells, have become one of the most interesting targets for the study of tissue regeneration because of their plasticity. BMSCs are multipotential cells that contribute to the regeneration of tissues such as bone, cartilage, fat, and muscle, and to the expression of many cytokines and cellular factors (Pittenger et al., 1999; Deans and Moseley, 2000; Dormady et al., 2001; Jackson et al., 2001; Bhagavati and Xu, 2004). Several studies have shown the capacity of BMSC-derived elements to localize in the murine central nervous system (CNS) and peripheral nervous system (PNS), to integrate in these tissues, and to assume the morphology of some resident cells (Eglitis and Mezey, 1997; Brazelton et al., 2000; Mezey et al., 2000; Sanchez-Ramos et al., 2000; Lu et al., 2001; Akiyama et al., 2002; Hudson et al., 2004). Given the pluripotency of BMSCs, the prospect of using them to elicit neuroprotection has been explored in spinal cord injury (Akiyama et al., 2002), traumatic brain injury (Lu et al., 2001), and peripheral nerve injury (Dezawa et al., 2001; Cuevas et al., 2002, 2004; Mimura et al., 2004). Cell replacement, trophic factor production, extracellular matrix molecule synthesis, guidance, remyelination, microenvironmental stabilization, and immune modulation have recently been proposed as beneficial mechanisms after cell implantation. Evidence suggests that BMSCs comprise a heterogeneous population of cells, including those with small rounded, large flattened and fibroblast-like morphology, with distinct plasticity and characterization (Sanchez-Ramos et al., 2000; Hudson et al., 2004). In the present study, our purpose was to elicit the functional recovery in peripheral nerve injury over a 15 mm gap defect by fibroblast-like BMSC implantation and to evaluate the kinetics of trophic factor expression during the regenerative process.

Materials and methods

Cell preparation

BMSCs were prepared from adult female Sprague–Dawley rats and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) as described previously (Cuevas et al., 2004). Briefly, after the dissociated cells were incubated for 24 h, the non-adherent cells were removed. The adherent cells were continuously cultured and then used for the experiments.

Differentiation studies

BMSCs with fibroblast-like morphology were cultured to confluence and changed to osteogenic medium (α-MEM supplemented with 10% FBS, 0.1 μM dexamethason, 10 mM β-glycerol phosphate, 50 μM ascorbate) and adipogenic medium (α-MEM supplemented with 10% FBS, 1 μM dexamethason, 5 μg/mL insulin, 0.5 mM isobutylmethylxanthine and 60 μM indomethacin) for 3 weeks. The differentiation potential for osteogenesis was assessed by the mineralization of calcium deposits by von Kossa histochemical staining. The production of intracellular lipid droplets was detected by Oil Red O staining for the evaluation of adipogenesis (Tsai et al., 2004).

Immunocytochemical analysis

Immunocytochemical detection was performed as previously reported (Chen et al., 2004a). Briefly, cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton-X 100. The cells were blocked with 5% nonfat milk for 30 min and then incubated with primary antibody against fibronectin (Sigma Chemical, 1:500) overnight at 4 °C. After washing, the cells were incubated with horseradish peroxidase-conjugated secondary antibody (1:500) for 1 h at room temperature. The color was developed with 3,3′-diaminobenzidine (Sigma Chemical) and observed under a light microscope.

Surgery

The animal study protocol was approved by the Animal Experimental Committee of Taichung Veterans General Hospital. Forty adult male Sprague–Dawley rats (200–250 g), divided into BMSC-treated ($n=20$) and control ($n=20$) groups, were anesthetized i.p. with sodium pentobarbital (50 mg/kg) during all surgical procedures. After skin incision, the sciatic nerve was exposed using a muscle splitting incision. With the aid of an operation microscope, the right sciatic nerve was severed and removed (15 mm) near the obturator tendon in mid-thigh (Chen et al., 2001). A 20-mm silicone tube (1 mm inner diameter, 2 mm outer diameter) was interposed into this nerve gap (Chen et al., 2000). Both proximal and distal nerve stumps were anchored into the conduit with 9-0 nylon microsutures. Most transplantation studies were carried out by suspending cells with crucial supporting substances such as collagen, laminin, fibronectin, or Matrigel (Chen et al., 2000). To get further insights into the effect of implanted cells, we used relatively inert substance, gelatin, as a suspending matrix. Mixtures of 2% gelatin (control group) or BMSCs (1×10^6 cells/tube) suspended with 2% gelatin (BMSC group) were then injected into the lumen of the conduit, using a syringe. The wound was subsequently closed in layers using 4-0 Dexon sutures. The rats were given food and water *ad libitum*. Four animals in each group were sacrificed 3 days after surgery and the rest of the animals were sacrificed 10 weeks after surgery.

Functional assessment

Functional evaluation of sciatic nerve regeneration was expressed by the sciatic function index (SFI) (Mimura et al., 2004). A technical assistant who was blinded to treatment