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Mesenchymal stromal cells prolong the lifespan in a rat model of amyotrophic lateral sclerosis

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Abstract

Background aims. Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by the loss of brain and spinal cord motor neurons (MN). The intraspinal and systemic grafting of mesenchymal stromal cells (MSC) was used to treat symptomatic transgenic rats overexpressing human superoxide dismutase 1 (SOD1) in order to alleviate the disease course and prolong the animals' lifespan. *Methods.* At the age of 16 weeks (disease onset) the rats received two grafts of MSC expressing green fluorescent protein (GFP^+ MSC) on the same day, intraspinally (10^5 cells) and intravenously (2×10^6 cells). Sham-treated animals were injected with phosphate-buffered saline (PBS). Motor activity, grip strength and body weight were tested, followed by immunohistochemical analysis. *Results.* The combined grafting of MSC into symptomatic rats had a significant effect on motor activity and grip strength starting 4 weeks after transplantation. The lifespan of animals in the treated group was 190 ± 3.33 days compared with 179 ± 3.6 days in the control group of animals. Treated rats had a larger number of MN at the thoracic and lumbar levels; these MN were of larger size, and the intensity of terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick-end labeling (TUNEL) staining in the somas of apoptotic MN at the thoracic level was much lower than in sham-treated animals. Transplanted GFP^+ MSC survived in the spinal cord until the end stage of the disease and migrated both rostrally and caudally from the injection site. *Conclusions.* Intraspinal and intravenous transplantation of MSC has a beneficial and possibly synergistic effect on the lifespan of ALS animals.

Key Words: *amyotrophic lateral sclerosis, human superoxide dismutase 1, mesenchymal stromal cells, motor neurons, neurodegenerative diseases, stem cells*

Introduction

Amyotrophic lateral sclerosis (ALS) is a rapidly progressing degenerative disease that selectively attacks motor neurons (MN) in the cortex, brain stem and spinal cord, leading to weakness, muscle atrophy, fasciculations, spasticity, paralysis and eventually death within 3–5 years after the onset of its clinical symptoms (1). Ninety per cent of all disease cases are considered to be sporadic, while the remaining 5–10% suffer from familial ALS caused by different genetic mutations [human superoxide dismutase 1 (SOD1), Alsin, SETX, TARDBP, etc.]. Of these familial cases, approximately 20% are caused by mutations in the gene encoding SOD1 located on chromosome 21q, i.e. approximately 2% of all cases of ALS (2); the corresponding protein is known to detoxify potentially cell-damaging free radicals (3). Similarly, transgenic mice and rats overexpressing mutant human SOD1 develop an age-dependent degeneration of MN, leading to paralysis and death, and thus are a suitable model for ALS (4). Effective treatments for this devastating disease have evaded researchers for many years. Now there is encouraging new evidence that intrathecal, intracerebroventricular or intramuscular delivery of various growth factors, such as insulin-like growth factor (IGF) (5) and vascular endothelial growth factor (VEGF) (6–8), leads to an improvement in disease progression and overall survival in a rat model of ALS. Another strategy for the treatment of ALS is stem cell transplantation (9–11). Stem cells could either replace or protect MN because of their ability to release neurotrophic factors that exert a direct influence on dying neurons or that indirectly influence the neuronal environment (12,13). Bone

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marrow (BM) mesenchymal stromal cells (MSC) possess unique immunologic and survival properties for cellular therapy: they are immunopotential, do not stimulate alloreactivity and escape lysis by cytotoxic T cells and natural killer (NK) cells (14–16). In addition, they induce the secretion of several growth factors by host cells, such as brain-derived neurotrophic factor (BDNF), neural growth factor (NGF) and IGF-1 (17,18). In the current study, we used a combined injection (intraspinal and intravenous) of MSC with the aim of evaluating its effect in animals that had already started to show the first symptoms of ALS. We examined motor activity [Basso, Beattie and Bresnaham (BBB) locomotor rating scale (19)], motor function (grip strength test), body weight, the number of MN, apoptosis and prolongation of lifespan.

Methods

Animals

Transgenic male hemizygous 7-week-old NTac:SD-Tg(SOD1–G93A)L26H rats that overexpress human SOD1, carrying the Gly93–Ala mutation, were obtained from Taconic (Hudson, NY, USA). The animals were housed under standard laboratory conditions: a 12:12 h dark:light cycle, room temperature of 23°C, two rats in one cage, with food and water supply *ad libitum*. The animals were divided randomly into two groups: one group was treated with rat BM green fluorescent protein MSC (GFP^+ MSC) ($n = 11$) and one group was injected only with phosphate-buffered saline (PBS) ($n = 8$). Two animals from the treated group (approximately 10% of the total number of animals) were excluded from the study 11 days after cell implantation: one died, while the second animal was killed because of poor health, both as a result of a urinary bladder infection. Thus there were nine rats in the MSC-treated group and eight rats injected with PBS that were tested and included in the histologic and statistical analyzes. All rats were immunosuppressed with cyclosporine A (CsA), 10 mg/kg/day, 1 day prior to injection of cells or PBS and then until the end stage of the disease. This study was performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) regarding the use of animals in research, and was approved by the Ethics Committee of the Institute of Experimental Medicine, Academy of Sciences of the Czech Republic (ASCR), Prague, Czech Republic).

BM MSC preparation and transplantation

Isolation and culturing of rat GFP^+ MSC. GFP^+ MSC were obtained from transgenic Sprague–Dawley rats [SD-Tg(CAG0EGFP)CZ-004Osb], kindly provided by Dr M. Okabe (Osaka University, Osaka, Japan) (20), bred at the laboratory of Dr M. Marsala (University of California, San Diego, CA, USA) and subsequently sent to the Institute of Experimental Medicine ASCR and bred in our animal facility. The BM was taken from the femurs and tibias of 16-day-old male animals that were killed using CO_2. After cutting the epiphysis, the BM was washed from the bones using a 2-mL syringe with a 21-gauge needle filled with Dullbecco's modified Eagle's medium with high glucose (DMEM), glutamax 15 μL/mL, 10% fetal calf serum (FCS) and primocin 2 μL/mL (complete medium). The BM was gently dissociated and then plated on Petri dishes. The medium was changed after 24 h. When cells reached 75–90% confluence, they were detached by trypsin/EDTA treatment and transferred into 75-cm^2 cell culture flasks. MSC from passage 4 were used for implantation. Prior to implantation, the cultures were checked for the cells' ability to differentiate into adipogenic, osteogenic and chondrogenic phenotypes (21).

Cell implantation

When the cells reached 90% confluence, they were harvested and washed with PBS. The volume was adjusted to 1 μL to count the cells. For transplantation, we used MSC from passage 4 (approximately 28 days of *in vitro* cultivation). Viability was assessed by the trypan blue method, and more than 90% of the cells were alive. GFP^+ MSC for intraspinal implantation were suspended at a concentration of 5×10^4 cells/1 μL PBS. For intravenous injection MSC were used at a concentration of 2×10^6/0.5 mL PBS. At the age of approximately 16 weeks (114 ± 5 days) the rats were anesthetized by isofluorane (3%) vapor inhalation in air and each animal received two intraspinal grafts, containing a total of 10^5 cells. The spinal cord was exposed at the T10 level, followed by one injection of MSC on the left and one on the right side (diagonally), with a distance between the injection sites of 3 mm. The cells were injected into the ventral horns of the spinal cord (at a depth of 1 mm from the dorsal surface, 1 mm laterally from the midline). The injections were made at a rate of 1 μL/min using a Nano-Injector (Stoelting Co., Wood Dale, IL, USA) by means of a glass pipette. The pipette was kept inside the tissue for 1 min, thus preventing the transplanted cell from leaking out of the host tissue. After the first procedure was completed and all the layers (muscles and skin) were closed by simple interrupted sutures, the animals underwent the second procedure of cell transplantation into the femoral vein. The skin was cut in the inner thigh region. The femoral vein was carefully isolated and exposed from the femoral