Cytotherapy, 2014; 0: 1–14

International Society for Cellular Therapy
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Human mesenchymal stromal cell transplantation modulates neuroinflammatory milieu in a mouse model of amyotrophic lateral sclerosis

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Abstract

Background aims. Mesenchymal stromal cells (MSCs), after intraparenchymal, intrathecal and endovenous administration, have been previously tested for cell therapy in amyotrophic lateral sclerosis in the SOD1 (superoxide dismutase 1) mouse. However, every administration route has specific pros and cons. *Methods.* We administrated human MSCs (hMSCs) in the cisterna lumbaris, which is easily accessible and could be used in outpatient surgery, in the SOD1 G93A mouse, at the earliest onset of symptoms. Control animals received saline injections. Motor behavior was checked starting from 2 months of age until the mice were killed. Animals were killed 2 weeks after transplantation; lumbar motoneurons were stereologically counted, astrocytes and microglia were analyzed and quantified after immunohistochemistry and cytokine expression was assayed by means of real-time polymerase chain reaction. *Results.* We provide evidence that this route of administration can exert strongly positive effects. Motoneuron death and motor decay were delayed, astrogliosis was reduced and microglial activation was modulated. In addition, hMSC transplantation prevented the downregulation of the anti-inflammatory interleukin-10, as well as that of vascular endothelial growth factor observed in saline-treated transgenic mice compared with wild type, and resulted in a dramatic increase in the expression of the anti-inflammatory interleukin-13. *Conclusions.* Our results suggest that hMSCs, when intracisternally administered, can exert their paracrine potential, influencing the inflammatory response of the host.

Key Words: *cell therapy, cisterna lumbaris, cytokines, microglia, motoneuron*

Introduction

Amyotrophic lateral sclerosis (ALS) is a late-onset neurodegenerative disease that causes degeneration and death of upper and lower motoneurons, leading to weakness, muscle atrophy, fasciculations, spasticity and finally, death as the result of respiratory failure (1). Currently, there is no treatment for ALS. Riluzole, the only drug approved by the Food and Drug Administration for ALS, has a very limited outcome because it increases survival by only 2–3 months compared with placebo (2).

In the past decade, stem cell therapy emerged as a possible strategy to modulate the motoneuron environment, in terms of astrogliosis and microglial activation that occur in ALS, and to deliver trophic factors to support motoneuron survival (3,4). To this aim, several studies reported beneficial effects after transplantation of different types of stem cells in animal models of ALS: mesenchymal stromal cells (MSCs) (5–7), neural stem cells (8–11), olfactory ensheathing cells (12) and induced pluripotent cells (13); also, clinical trials have demonstrated the feasibility of human MSC (hMSC) transplantation in patients (14–16). The cell type that better matches safety conditions and immunomodulatory/neurotrophic roles consists of MSCs. MSCs are bone marrow (BM) cells expanded *ex vivo* (17); they represent a small fraction (0.001–0.01%) of the BM

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(Received 22 October 2013; accepted 9 February 2014)

http://dx.doi.org/10.1016/j.jcyt.2014.02.003

cell population. Although BM is the best characterized source of MSCs, umbilical cord blood, Wharton's jelly, placenta, adipose tissue and many others represent promising alternatives (18). Several groups, including ours (6), have shown that MSC transplantation can delay the behavioral symptoms and motoneuron death in animal models of ALS (4). MSCs have been delivered intraparenchymally, intrathecally, intramuscularly or intravenously, each of which has its own advantages and disadvantages (4). In the present study, we decided to administer MSCs in the cisterna lumbaris, which is easily accessible and could be used in outpatient surgery, in an animal model of ALS, the SOD1 (superoxide dismutase 1) G93A mouse, at the earliest onset of symptoms. We provide evidence that this route of administration can exert positive effects. Additionally, we analyzed the expression of several pro-inflammatory and anti-inflammatory cytokines by the host, identifying one of the hMSC mechanisms of action.

Methods

Animal care and use

Experiments were performed on male transgenic mice B6SJL-TgN(SOD1G93A)1Gur over-expressing human SOD1, containing the Gly93 to Ala mutation (Jackson Laboratory, Bar Harbor, ME, USA; stock No. 002726); these mice have a high transgene copy number, as reported in the data sheet. Founders were kindly gifted by M. Bentivoglio and R. Mariotti (University of Verona). The colony was derived by breeding of male transgenic mice to naive (B6xSJL/J)F1 female mice (Janvier SAS, Le Genest-Saint-Isle, France).

All experimental procedures on live animals were carried out in strict accordance with the European Communities Council Directive 86/609/EEC (November 24, 1986) Italian Ministry of Health and University of Turin institutional guidelines on animal welfare (law 116/92 on Care and Protection of living animals undergoing experimental or other scientific procedures; authorization No. 17/2010-B, June 30, 2010); additionally, an ad hoc Ethical Committee of the University of Turin approved this study. All efforts were made to minimize the number of animals used and their suffering. They were identified by polymerase chain reaction (PCR) according to Jackson Laboratory's genotyping protocol.

Genotyping mice

DNA from mouse tail was extracted by incubation of a 0.5-cm-long specimen of tail in 100 μL of lysis buffer (10 mmol/L Tris HCl, 50 mmol/L KCl, 0.01% gelatin, 0.45% IGEPAL® CA-630 [Sigma-Aldrich], 0.4% Tween-20) and 25 μg of proteinase K at 55°C overnight under gentle shaking. On the extracted DNA, we performed PCR to evaluate the presence of the human transgene superoxide dismutase-1 (hSOD1). The primers used, suggested by Jackson Laboratories, were 5′-CATCAGCCCTAATCCATCTGA-3′ and 5′-CGCGACTAACAATCAAAGTGA-3′ for *hSOD1* gene and 5′-CTAGGCCACAGAATTGAAAGATCT-3′ and 5′-GTAGGTGGAAATTCTAGCATCATCC-3′ for mouse interleukin 2 gene (*mIL-2*), as internal control.

Behavioral tests

To treat the animals at the symptom onset, the mice (hMSC TG, n = 16; sal TG, n = 14) were weighed weekly and underwent a battery of behavioral tests starting from the asymptomatic phase: scoring of motor deficits by a trained observer (6), rotarod and paw grip endurance (PaGE) tests (19).

The first 2 weeks of tests (starting around postnatal day 60 [P60]) were considered as training for the animals that were tested weekly. Thereafter, the tests were performed twice per week.

The values obtained before onset of symptoms were considered as baseline to be compared with those obtained after treatment until the mice were killed at 14 days after grafting, to evaluate the effects of hMSCs on the decay in behavioral performance caused by disease.

The neurological test was performed in an open field [size: 70 (width) × 120 (depth) cm] to assess gait; the mice were evaluated for signs of motor deficits with the following -point scoring system: 4 points if normal (no sign of motor dysfunction); 3 points if hind limb tremors were evident when suspended by the tail; 2 points if gait abnormalities were present; 1 point for dragging of at least one hind limb; 0 points for inability to right itself within 30 seconds.

For the rotarod test, we measured the time animals could remain on the rotating cylinder in a 7650 accelerating model of a rotarod apparatus (Ugo Basile, Comerio, Va, Italy). Each animal was given three trials. The arbitrary cut-off time was 300 seconds, and the accelerated speed went from 4 to 32 rpm. The size of rotarod equipment is 40 (width) × 30 (depth) × 38 (height) cm, and the rotating cylinder diameter is 3 cm.

For the PaGE test, the animal was placed on the wire lid of conventional housing cage [size: 38 (width) × 22 (depth) cm]. The lid was gently shaken to prompt the mouse to hold onto the grid before it was swiftly turned upside down. Grip score was