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Research article

Transplantation of bone marrow mononuclear cells prolongs survival, delays disease onset and progression and mitigates neuronal loss in pre-symptomatic, but not symptomatic ALS mice

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HIGHLIGHTS

- We investigate the effect of bone marrow mononuclear cells (BMMC) in ALS mice.
- Pre-symptomatic BMMC transplantation improves the animal's clinical condition.
- Only non-mSOD1 BMMC are beneficial in late symptomatic animals.
- Early administration of BMMC promotes better outcomes.

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ABSTRACT

Cell-based therapy provides a novel strategy to restore lost neurons or modulate the degenerating microenvironment in amyotrophic lateral sclerosis (ALS). This study verified the therapeutic potential of bone marrow mononuclear cells (BMMCs) in SOD1^{G93A} mice. BMMCs were obtained from enhanced green fluorescent protein (EGFP) transgenic C57BL/6 mice (^{EGFP}BMMCs) or from SOD1^{G93A} transgenic mice (^{mSOD1}BMMCs) and given to mice at the pre-symptomatic or late symptomatic stage. Survival, body weight and motor performance data were recorded. DNA integrity was evaluated using the alkaline comet assay. The spinal cords were collected to assess motoneuron preservation and cell migration. ^{EGFP}BMMCs and ^{mSOD1}BMMCs transplantation to pre-symptomatic SOD1^{G93A} mice prolonged survival and delayed disease progression. The effects were more significant for the ^{EGFP}BMMC-transplanted mice. In late symptomatic mice, ^{EGFP}BMMCs promoted a discrete increase in survival, without other clinical improvements. DNA from ^{EGFP}BMMCs and ^{mSOD1}BMMCs was found in the spinal cords of transplanted animals. DNA damage was not modified by BMMCs in any of the studied groups. Despite positive behavioral effects observed in our study, the limited results we observed for late transplanted mice call for caution before clinical application of BMMCs in ALS.

Abbreviations: ALS, amyotrophic lateral sclerosis; BMMCs, bone marrow mononuclear cells; ChAT, choline acetyltransferase; EGFP, enhanced green fluorescent protein; ^{EGFP}BMMC-70, SOD1^{G93A} mice transplanted at 70 days with BMMCs from C57BL/6-EGFP mice; ^{EGFP}BMMC-110, SOD1^{G93A} mice transplanted at 110 days with BMMCSs from C57BL/6-EGFP mice; GLT1, glutamate transporter 1; MSC, mesenchymal stem cells; ^{mSOD1}BMMC-70, SOD1^{G93A} mice transplanted at 70 days with BMMCs from SOD1^{G93A} mice; ^{mSOD1}BMMC-110, SOD1^{G93A} transplanted at 110 days with BMMCs from SOD1^{G93A} mice; SAL, SOD1^{G93A} mice treated with saline; TNF, αtumor necrosis factor alpha.

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1. Introduction

Cell therapy emerged as a promising approach to treat neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). A number of strategies have been studied, such as the transplantation of genetically modified cells to secrete trophic factors [1], human umbilical cord blood cells [2,3], neural stem cells [4], iPSC-derived cells [5], mesenchymal stem cells (MSC) [6–8] and other cellular populations of the bone marrow [9,10], including bone marrow mononuclear cells (BMMCs) [11–14]. Nevertheless, the reported efficacy from clinical studies is still controversial [15]. Even if the procedure has been shown to be safe, more preclinical studies are required to determine the best cell source and dosage, the route of administration, the use of autologous or heterologous donors and the time of intervention.

It has been reported that BMMCs promote beneficial effects in pre-symptomatic or early symptomatic SOD1 mice [11–14,16]. However, the therapeutic potential of intravenously transplanted BMMCs in late symptomatic ALS mice remains unknown. Furthermore, additional studies are needed to clarify aspects related to the transplantation from mutant donors. To explore these possibilities, we intravenously injected SOD1^{G93A} mice with BMMCs from enhanced green fluorescent protein (EGFP) transgenic C57BL/6 mice or SOD1^{G93A} donors. Transplantation was performed in either pre-symptomatic or late symptomatic mice bearing the SOD1^{G93A} transgene.

2. Material and methods

2.1. Animals

B6SJL-Tg(SOD1-G93A)1Gur/J mice breeding pairs were purchased from the Jackson Laboratory (Bar Harbor, ME, EUA), and the offspring were genotyped as previously described [17,18]. Transgenic EGFP^{+} C57BL/6 mice were also used in this study. All animals were kept under 12-h light/dark cycle, 22–24 °C temperature and given free access to food and water. The Animal Care and Ethics Committee at PUCRS approved this study (09/00091). The experimental groups were balanced with regard to animal gender, age and body weight. Care was taken distributing littermates among the different experimental groups so that the number of copies of the mutant SOD1 gene would likely be similar.

2.2. BMMC preparation

BMMCs were obtained from EGFP^{+} C57BL/6 or from SOD1^{G93A} male mice, as previously described [19]. Either EGFP or the Y chromosome was used as a reporter for the transplanted cells. BMMCs were incubated with conjugated antibodies against CD34, CD19, CD117, CD45 and Sca1. The labeled cells were collected and analyzed using a FACSCalibur cytometer.

2.3. BMMC transplantation

The mice were distributed into five experimental groups: (1) the ^{EGFP}BMMC-70 group: SOD1^{G93A} mice transplanted at 70 days (pre-symptomatic) with BMMCs from C57BL/6-EGFP mice (n = 15); (2) the ^{mSOD1}BMMC-70 group: SOD1^{G93A} mice transplanted at 70 days (pre-symptomatic) with BMMCs from SOD1^{G93A} mice (n = 15); (3) the ^{EGFP}BMMC-110 group: SOD1^{G93A} mice transplanted at 110 days (late symptomatic) with BMMCSs from C57BL/6-EGFP mice (n = 15); (4) the ^{mSOD1}BMMC-110 group: SOD1^{G93A} transplanted at 110 days (late symptomatic) with BMMCs from SOD1^{G93A} mice (n = 14); and (5) the SAL group: SOD1^{G93A} mice treated with saline (n = 23). ^{EGFP}BMMCs or ^{mSOD1}BMMCs suspensions were prepared at a concentration of 1×10^7, and injected via tail vein. An additional cohort of animals (n = 2 per transplantation group) received BMMCs as described above but was sacrificed at 24 h, 1 week or 2 weeks after transplantation, and tissue samples were collected for PCR. Other animal cohorts were treated as described and euthanized at 120 days for histological analysis (n = 5 per group) or for the alkaline comet assay (n = 4 per group).

2.4. Survival

Endpoint was determined as the age when the mouse was unable to right itself within 30 s when placed on its back in a supinated position, as previously described [10].

2.5. Clinical evaluation

Body weight measurements were used to determine disease onset and the symptomatic stage. All mice were weighed weekly beginning on the 9th week. When the mice were 11 weeks old, they were weighed twice a week, and three times a week when the mice reached the age of 16 weeks, until the endpoint. All measurements were taken between 1 and 4 p.m. [20]. Disease onset was defined as the maximum weight recorded for each animal retrospectively. The animals were considered to have reached the symptomatic stage once they had lost 10% of their maximum weight [10]. The difference between the age at onset and the age at endpoint was used as a measure of disease progression.

2.6. Motor performance

Rotarod (EFF 412, Insight, Brazil) tests were conducted weekly from 11 weeks of age to endpoint. The mouse had up to seven minutes to remain in the rotating cylinder at 16 rpm. Mice were placed in the direction opposite to the rotation. The animals using paws to hold themselves to the cylinder for two consecutive laps were excluded. The latency until the mouse dropped from the cylinder was counted.

2.7. Polymerase chain reaction (PCR)

Nested PCR was used to identify the presence of BMMCs in the tissues of transplanted animals, as previously described [19]. To detect BMMCs from SOD1^{G93A} donors, who do not express EGFP, the samples were collected from female recipients, and the Y chromosome was detected. Tissue samples were collected from the spinal cord (dorsal root ganglions were removed from tissue), muscle, liver, spleen, lungs and heart at 1, 7 and 14 days after transplantation, and at endpoint. For EGFP DNA detection, groups were gender balanced.

2.8. Determination of DNA damage

To evaluate whether BMMCs prevent or mitigate the oxidative stress associated with the SOD1^{G93A} mutation [21], the alkaline comet assay was performed. Samples of the spinal cord, muscle, cerebellum and cerebral cortex from the groups SAL, ^{EGFP}BMMC-70 and ^{EGFP}BMMC-110 were collected and processed, as previously described [22].

2.9. Immunofluorescence

Transverse sections (20 μm) of the lumbar spinal cord were incubated overnight with a primary rabbit anti-ChAT (choline acetyltransferase) antibody (1:500; Abcam, Cambridge, MA, USA). Alexa Fluor 568 goat anti-rabbit IgG (H + L) was used as secondary antibody (1:1.000; Invitrogen, Carlsbad, CA, USA). Glass coverslips were mounted with DAPI (4′,6-diamidino-2-phenylindole) using