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BRAIN RESEARCH

Research Report

Therapeutic window for treatment of cortical ischemia with bone marrow-derived cells in rats

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ABSTRACT

The beneficial effect of treatment with bone marrow mononuclear cells (BMMCs) was evaluated in different therapeutic windows in a rat model of focal ischemia induced by thermocoagulation of the blood vessels in the left motor, somestesic, and sensorimotor cortices. We also compared the therapeutic benefits between BMMCs and bone marrow-derived mesenchymal stem cells (MSCs). BMMCs and MSCs were obtained from donor rats and injected into the jugular vein after ischemia. BMMCs-treated animals received approximately 3×10^7 cells at post-ischemic days (PIDs) 1, 7, 14, or 30. MSCs-treated animals received approximately 3×10^6 cells at PIDs 1 and 30. Control animals received only the vehicle. The animals were then evaluated for functional sensorimotor recovery weekly with behavioral tests (cylinder test and adhesive test). Significant recovery of sensorimotor function was only observed in the cylinder test in animals treated with BMMCs at PIDs 1 and 7. Similar effects were also observed in the animals treated with MSCs 1 day after ischemia, but not in animals treated with MSCs 30 days after ischemia. Significant decrease in glial scarring did not seem to be a mechanism of action of BMMCs, since treatment with BMMCs did not change the level of expression of GFAP, indicating no significant change in the astrocytic scar in the periphery of the ischemic lesion. These results suggest that BMMCs might be an efficient treatment protocol for stroke only in the acute/subacute phase of the disease, and its efficiency in inducing functional recovery is similar to that of MSCs.

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Abbreviations: ANOVA, analysis of variance; BMMCs, bone marrow mononuclear cells; CNS, Central Nervous System; CXCR4, chemokine (CXC motif) receptor 4; CY5, cyanine 5; ECL, enhanced chemiluminescence; FC, flow cytometry; FITC, fluorescein isothiocyanate; GFAP, glial fibrillary acidic protein; HRP, horseradish peroxidase; ISCT, International Society for Cellular Therapy; MCAO, middle cerebral artery occlusion; MSCs, mesenchymal stem cells; OD, optical density; PBS, phosphate-buffered saline; PE, phycoerythrin; PID, post-ischemic day; SDF-1, stromal cell-derived factor-1; SEM, standard error of the mean; TW, therapeutic window

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1. Introduction

Bone marrow-derived cells have been described as multipotent, able to be chemoattracted to lesioned tissues, and capable of releasing many cytokines and trophic factors (Krause et al., 2001; Chopp and Li, 2002; Crain et al., 2005; Shyu et al., 2006; Kawamoto and Losordo, 2008). Several studies have shown beneficial effects of treatment with these cells in animal models of ischemia and also in a few clinical trials of ischemic stroke, suggesting that this therapeutic strategy is promising (Chopp and Li, 2002; Bang et al., 2005; Mezey, 2007). The cells used in these studies were bone marrow-derived mesenchymal stem cells (MSCs), a population of cells obtained after culturing bone marrow aspirate for weeks. Alternatively, a few studies have used bone marrow mononuclear cells (BMMCs), a fraction of the bone marrow that contains two types of adult stem cells (MSCs and hematopoietic stem cells) (Orkin, 2000; Weissman et al., 2001), hematopoietic progenitor cells (Weissman et al., 2001), and endothelial progenitor cells (Wang et al., 2008), and has also been shown to be beneficial in animal models of stroke (Iihoshi et al., 2004; Kamiya et al., 2008; Giraldi-Guimarães et al., 2009). The feasibility and safety of autologous BMMCs transplantation has also been evaluated in a clinical trial in patients with ischemic stroke (Mendonça et al., 2006; Mendez-Otero et al., 2007).

Most of the studies described up to now have used bone marrow-derived cells in the acute or subacute phase of ischemia, and only a few of them have shown significant functional recovery when MSCs were administered 1 month after ischemia (Shen et al., 2007), suggesting that this treatment could be also effective when performed in the chronic phase of the disease.

We recently demonstrated functional recovery after treatment with BMMCs 1 day post-ischemia (Giraldi-Guimarães et al., 2009). In the present study, we evaluated the therapeutic window of treatment with BMMCs in a model of sensorimotor cortical ischemia. In addition, we compared the therapeutic benefits of BMMCs and MSC, since in a clinical setting it would be easier to use BMMCs rather than MSC.

2. Results

As previously described (Szele et al., 1995; Giraldi-Guimarães et al., 2009), our procedure of thermocoagulation induced a consistent ischemic lesion that included the six cortical layers, sparing the white matter (data not shown).

2.1. *Effects of BMMCs transplantation on sensorimotor function in different therapeutic windows*

We have previously demonstrated considerable recovery of sensorimotor function after transplantation of BMMCs 1 day after ischemia in the cylinder test (Giraldi-Guimarães et al.,

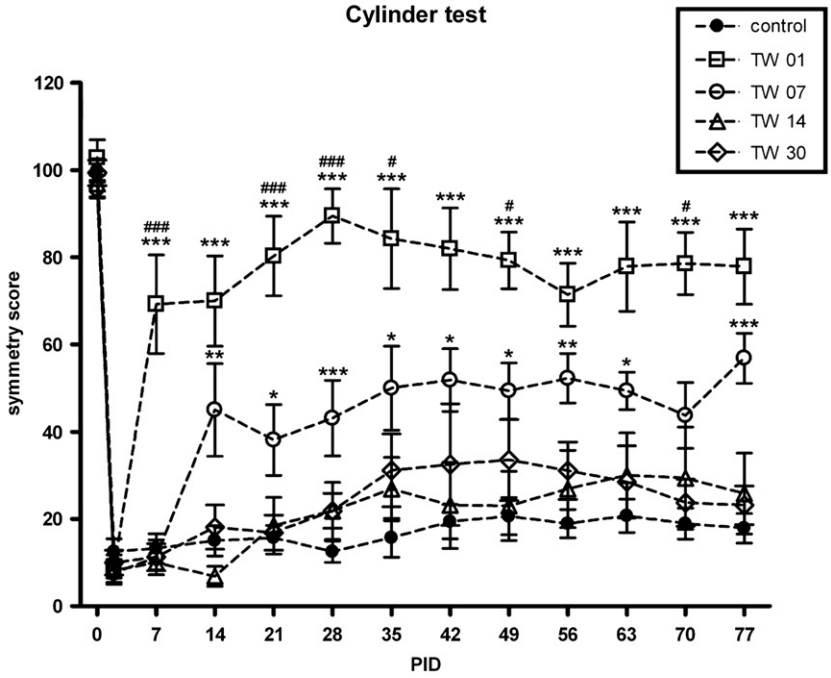

Fig. 1 – Evaluation of different therapeutic windows after treatment with BMMCs in the cylinder test. The analysis of the sensorimotor function in the cylinder test showed a significant recovery of the impaired forelimb only in the TW 01 and TW 07 groups. Before the induction of the lesion, all groups showed symmetrical use of the forelimbs, with symmetry scores close to 100. This test was performed 1 day before the thermocoagulation, but was plotted on the graph as PID 0. As expected, all groups showed clear asymmetry in the use of the forelimbs at PID 1, preferentially using the unimpaired limb. On the following analyzed PIDs, only the TW 01 and TW 07 groups showed a return to a more symmetrical use of the forelimbs, indicating recovery of function of the impaired limb. However, the recovery was greater in the TW 01 group. TW 01 group, $n=7$; TW 07 group, $n=8$; TW 14 group, $n=8$; TW 30 group, $n=8$; control group, $n=14$. Data shown in the graph are means ± S.E.M. * represents comparison with the control group and $^{\#}$ represents comparison with the TW 07 group. * or $^{\#}=p<0.05$, $^{**}=p<0.01$, *** or $^{\#\#\#}=p<0.001$; Tukey *post-hoc* test.