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Research Report

Bone-marrow mononuclear cells reduce neurodegeneration in hippocampal CA1 layer after transient global ischemia in rats

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ABSTRACT

Global cerebral ischemia (GCI) results in death of the pyramidal neurons in the CA1 layer of the hippocampus. In this study we used the four-vessel occlusion (4VO) model of GCI to investigate a potential neuroprotective role of bone-marrow mononuclear cells (BMMCs) transplantation. BMMCs (3×10^7) were injected through the carotid artery, 1 or 3 days after ischemia (DAI), and the number of cells undergoing degeneration was investigated in brains at 7 DAI. A significant decrease in the number of dying cells was observed in the treated group, compared to animals treated with saline. Biodistribution of the injected cells (1 or 3 DAI) was investigated by ^{99m}Technetium labeling of the BMMCs and subsequent image analysis 2 h after transplantation. In addition, the presence of CellTrace™-labeled BMMCs was investigated in tissue sections of the hippocampal area of these transplanted animals. BMMCs treatment significantly reduced the number of FJ-C positive cells in the hippocampal CA1 layer at 7 DAI. We also observed a decrease in the number of activated microglia/macrophage (ED1-positive cells) in the BMMCs-treated group compared with the untreated group. Our data show that BMMCs are able to modulate the microglial response and reduce neurodegeneration in the CA1 layer.

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1. Introduction

In the United States, 300,000–450,000 individuals suffer cardiac arrest each year (Callans, 2004), the main cause for global cerebral ischemia. More than half of the patients who survive have permanent brain impairment of variable grades (Pusswald et al., 2000). The brain injury caused by transient global cerebral ischemia is characterized by a delayed selective death of CA1 pyramidal neurons in the hippocampus (Kirino, 1982; Pulsinelli and Brierley, 1979). This region is critically involved in spatial learning and memory, and the degeneration of pyramidal neurons results in impairment of these functions (Bendel et al., 2005; Milani et al., 1998).

Pathophysiological mechanisms in cerebral ischemia include anaerobic glycolysis, membrane depolarization, glutamate excitotoxicity, ion imbalance, oxidative stress, activation of proteases, and inflammation (Harukuni and Bhardwaj, 2006; Rodrigo et al., 2005). These events precede and contribute to the death of hippocampal CA1 neurons, which can be detected three days after global ischemia (Block, 1999; Kirino, 1982). The inflammatory response after global cerebral ischemia involves, among other features, reactive microgliosis in and around the damaged area of the brain, which can be observed from the first days after reperfusion (Sugawara et al., 2002; Yasuda et al., 2011). This process is characterized by overactivation of the microglia (the resident innate immune cells in the brain), and plays an important role in the progression of the ischemic injury through the release of cytokines and cytotoxic molecules (Block et al., 2007).

Although some studies have shown beneficial effects of microglial activation (Lalancette-Hebert et al., 2007; Ohtaki et al., 2008), most reports indicate that modulation of microglial activation reduces the neuronal death induced by cerebral ischemia (Franco et al., 2012; Hirko et al., 2008; Kaundal and Sharma, 2011).

Stem cell therapy has been considered a potential therapeutic alternative for various pathologies of the central nervous system (CNS) (Hess and Borlongan, 2008; Park et al., 2010). In particular, the transplantation of bone marrow-derived cells has shown positive results in preclinical models of CNS lesions (Lee et al., 2010a; Levy et al., 2008; Zaverucha-do-Valle et al., 2011), including cerebral ischemia (Brenneman et al., 2010; de Vasconcelos Dos Santos et al., 2010; Giraldi-Guimaraes et al., 2009; Vasconcelos-dos-Santos et al., 2012). Although the mechanisms involved are still under discussion, many studies have demonstrated that transplantation of bone-marrow mononuclear cells (BMMCs) promotes neuroprotection and functional recovery of animals subjected to focal cerebral ischemia (Franco et al., 2012; Giraldi-Guimaraes et al., 2009; Yang et al., 2011). Our aim was to investigate whether similar effects could be observed in a model of global cerebral ischemia. To our knowledge, only a few studies have investigated the effect of bone-marrow mesenchymal stem cells in preclinical models of global cerebral ischemia (Garbayo et al., 2011; Ohtaki et al., 2008; Perasso et al., 2010; Wang et al., 2008; Zheng et al., 2010) and only one study showed the biodistribution of BMMCs but did not investigate the effects of cell transplantation in the neurodegeneration and reactive microgliosis, as shown in the present paper (Makela et al., 2013). The advantage of BMMCs is that they are easily obtained and do not need extensive culturing before use. In addition, they have proved to be safe in clinical studies of stroke (Battistella et al., 2011; Friedrich et al., 2012; Rosado-de-Castro et al., 2013a; Rosado-de-Castro et al., 2013b).

2. Results

2.1. Time course of neurodegeneration in the CA1 layer after 4VO

Different models of global cerebral ischemia can lead to different temporal patterns of injury in the CA1 layer of the hippocampus. In our model we investigated the time course of neuronal degeneration in the pyramidal layer of CA1, assessing the number of Fluoro-Jade C (FJ-C)-positive cells 3, 7 and 14 DAI after global cerebral ischemia. We observed an increase in the number of FJ-C-positive cells from 3 to 7 days after ischemia (from 17.1 ± 0.89 cells/mm at 3 DAI to 159.1 ± 22.8 cells/mm at 7 DAI). This number decreased in the following week (114.3 ± 16.5 cells/mm) ($p = 0.0001$; Fig. 1). We also observed the presence of FJ-C positive cells in the dentate gyrus as well as in other regions of the brain known to be vulnerable in this model of ischemia, such as the striatum and cerebral cortex (data not shown).

2.2. Characterization and biodistribution of the transplanted BMMCs

The cellular fraction obtained from the bone marrow after separation by density gradient in Histopaque 1083 was heterogeneous, with different cell populations. Analysis by flow cytometry showed that approximately 4% of the analyzed fraction consisted of hematopoietic precursor cells (CD45+ and CD34+); 8.03 ± 0.39% by monocytes (CD45+ and CD11b/c+); 48.8 ± 13.2% by granulocytes (CD45+/Granulocytes) and 35.9 ± 3.5% by immature hematopoietic stem cells (CD45+ and CD90 +).

To investigate the biodistribution and homing of the injected BMMCs, we labeled the cells with ^{99m}Technetium (^{99m}Tc) and injected these cells 1 or 3 DAI. The animals were examined by whole-body scintigraphic imaging 2 h after transplantation. We observed activity in the injection site (Fig. 2A and B: non-ischemic rats; C and D: ischemic rats injected 1 DAI; E and F: ischemic rats injected 3 DAI) and in the liver and spleen (Fig. 2G and H) but no activity was distinguished in the brain. After imaging, the animals (injected 3 DAI) were euthanized, and the brains isolated and analyzed by gamma-well counting. We observed a difference between non-ischemic and ischemic groups ($2.4 \pm 1.6 \times 10^{-2}$% ID/g vs. $11.8 \pm 6.5 \times 10^{-2}$% ID/g; $p = 0.0159$; Fig. 2I), suggesting that, although in very low quantity, global ischemic injury led to an increase in the uptake of ^{99m}Tc-BMMCs in the brain region. However, the short half-life of ^{99m}Tc did not allow us to follow the cells for more than 24 h after injection. In order to investigate the fate of the injected cells for longer periods, BMMCs were labeled with CellTrace™, transplanted 1 or 3 DAI and the presence of labeled cells in