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Research Report

A human neural stem cell line provides neuroprotection and improves neurological performance by early intervention of neuroinflammatory system

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ABSTRACT

A human neural stem cell line, HB1.F3, demonstrated neuroprotective properties in cerebral ischemia animal models. In this study, we have investigated about the mechanisms of such neuroprotection, mainly focusing on the neuroinflammatory system at an earlier time point of the pathology. Cerebral ischemia model was generated by middle cerebral artery occlusion (MCAO) in adult male Wister rats. HB1.F3 cells were transplanted through jugular vein 6 h after MCAO. Forty eight hours after MCAO, transplanted rats showed better neurological performance and decreased TUNEL positive apoptotic cell number in the penumbra. However, haematoxylin and eosin staining and immunostaining showed that, HB1.F3 cells did not affect the necrotic cell death. Twenty four hours after MCAO (18 h after HB1.F3 transplantation), infiltrated granulocytes and macrophage/microglia number in the core regions were decreased compared to PBS-treated controls. Immunohistochemical analysis further demonstrated that the transplantation decreased inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 expressing cell number in the core and penumbra, respectively. Double immunofluorescence results revealed that iNOS was mainly expressed in granulocytes and macrophage/microglia in the core region, and COX-2 mainly expressed in neurons, endothelial cells and granulocytes in penumbra. Further analysis showed that although the percentage of iNOS expressing granulocytes and macrophage/microglia was not decreased, COX-2 expressing neurons and vessel number was decreased by the transplantation. In vitro mRNA analysis showed that brain-derived neurotrophic factor (BDNF), basic fibroblast growth factor (βFGF) and bone

Abbreviations: iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenese-2; BDNF, brain derived neurotrophic factor; FGF, fibroblast growth factor; BMP-4, bone morphogenic protein-4; MCAO, middle cerebral artery occlusion

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morphogenic protein (BMP)-4 expression was high in cultured HB1.F3 cells. Thus, our results demonstrated that HB1.F3 cell transplantation provide neuroprotection possibly through the regulation of early inflammatory events in the cerebral ischemia condition.

1. Introduction

Cerebral ischemia or stroke is a leading cause of death and long term disability worldwide. It results from transient or permanent disruption of cerebral blood flow, leading to necrotic death of the brain tissue supplied by the affected artery (Kumar et al., 2010). Such event activates an inflammatory condition in the affected area, marked by infiltration of inflammatory cells (Hallenbeck, 1996; Wang et al., 2007; Zheng and Yenari, 2004). Infiltration of granulocytes starts at very early time point, within hours of the initiation of the process, and progressively increases up to 48 h (Barone and Feuerstein, 1999; Gronberg et al., 2013; Kumar et al., 2010; Wang et al., 2007). Phagocytic cells including resident and circulating macrophage/microglia infiltration and accumulation follow the granulocytes, which are usually evident after 48 h, and become prominent inflammatory cell type during following 2–3 weeks (Gronberg et al., 2013; Kumar et al., 2010). These accumulated inflammatory cells produce various type inflammatory factors including cytokines, chemokines and enzymes (del Zoppo et al., 2000; Doll et al., 2014), and also clear up the dead tissues (Woo et al., 2012). Such processes are vital for the reparative process that ensue the ischemic insult. On the other hand, inflammation can induce an apoptotic cell death in the transition region between necrotic and normal tissue, so called penumbra, for a fairly prolonged period of time (Villa et al., 2003). Hence, the mature infarct size is usually much bigger than necrotic brain tissue of affected artery supply area. Such brain tissue of penumbra that is 'at risk' of apoptotic cell death, is salvageable by proper interventions of apoptotic and inflammation processes (Barone, 2009).

In recent years, the management and treatment protocols for stroke have been evaluated and improved (Grossman and Broderick, 2013), yet that fall far behind with respect to the disease modifying and restorative capability. However, based on the remarkable advances about the understanding of stroke pathology, several potential targets have been identified and accordingly strategies are being developed and tested. Strategies such as control of neuroinflammation, regeneration of neural tissue by exogenous stem cell transplantation or stimulation of endogenous neurogenesis show promises regarding this matter (Chang et al., 2013; Sheikh et al., 2011; Taguchi et al., 2004). Interestingly, exogenous stem cell based studies not only demonstrated the homing ability of these cells to the lesion area, but also showed neuroprotective and neuroinflammation modulatory functions, along with being differentiated into neural tissue (Chang et al., 2013; Sheikh et al., 2011; Wakabayashi et al., 2010). Hence, stem cell transplantation is suggested to modulate most of the potential targets of stroke pathology. Several stem cell types including mesenchymal stem cells, neural stem cells (NSC), embryonic stem cells and induced pluripotent cells are being tested (Chen et al., 2010; Takahashi et al., 2008; Wakabayashi et al., 2010; Yanagisawa et al., 2006). Among these cell types, NSC-based therapy could be important because of its neuronal differentiation capability, along with the ability to enhance angiogenesis and endogenous neurogenesis, and modulation of neuroinflammatory system (Kim et al., 2008, 2009; Sheikh et al., 2011; Tang et al., 2014). Indeed, NSC transplantation has been found to improve functional neurological recovery in cerebral ischemia animal models (Kim et al., 2008; Takahashi et al., 2008; Tang et al., 2014). Although, NSC is shown to be differentiated into mature neurons in the lesion area of cerebral ischemia animal models, it is difficult for such neurons to integrate into the neural circuitry. Hence, immune modulation might be an important aspect for such beneficial effects of NSC transplantation.

Several cell transplantation studies demonstrated that the transplantation during subacute phase, about 24 h after middle cerebral artery occlusion (MCAO), provide better result (Hao et al., 2014; Song et al., 2011). Accordingly, immune modulatory effects of cell transplantation during subacute phase are being investigated extensively (Sheikh et al., 2011; Wang et al., 2013). However, intervention during very early phase and understanding the modulatory effects at that time might also be important because during this time the events of neuroinflammation and other pathological aspects of stroke are different than that of sub-acute phase (Gronberg et al., 2013; Kumar et al., 2010). Therefore, in this study we aimed to investigate the effects of a neural stem cell line (HB1.F3) transplantation during early phage on the pathological changes in a cerebral ischemia condition. We found that HB1.F3 cell line transplantation at an earlier time point affects the initial events of neuroinflammation at the level of cell infiltration and pro-inflammatory gene expression.

2. Results

2.1. *Effects of HB1.F3 transplantation on neurological performances, tissue damage and cellular apoptosis in MCAO rat brains*

The rats included in the study showed no neurological deficit prior to MCAO. Six hours after MCAO, animals having neurological deficit with NSS score between 10 and 12 were randomly divided into 3 groups. There was no significant difference in NSS score among the groups at this time point. Forty eight hours after MCAO (42 h after HB1.F3 transplantation), NSS assessment was done again. The results showed