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Research report

Effects of neural stem cell media on hypoxic injury in rat hippocampal slice cultures

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ABSTRACT

Neonatal hypoxic-ischemic brain injuries cause serious neurological sequelae, yet there is currently no effective treatment for them. We hypothesized that neurotrophic factors released into the medium by stem cells could supply hypoxia-damaged organotypic hippocampal slice cultures with regenerative abilities. We prepared organotypic slice cultures of the hippocampus of 7-day-old Sprague-Dawley rats based on the modified Stoppini method; slices were cultured for 14 days in vitro using either Gahwiler's medium (G-medium) or stem cell-conditioned medium (S-medium) as culture medium. At day 14 in vitro, hippocampal slice cultures were exposed to 95% N_2 and 5% CO_2 for 3 h to induce hypoxic damage, the extent of which was then measured using propidium iodide fluorescence and immunohistochemistry images. We performed dot blotting to estimate neurotrophic/growth factor levels in the G- and S-media. Organotypic hippocampal slices cultured using S-medium after hypoxic injury were significantly less damaged than those cultured using G-medium. GLUT1, NGF, GDNF, VEGF, GCSF, and IGF2 levels were higher in S-medium than in G-medium, whereas FGF1, HIF, and MCP3 levels were not significantly different between media. In conclusion, we found that stem cell-conditioned medium had a neuroprotective effect against hypoxic injury, and that, of the various neurotrophic factors in S-medium, NGF, GDNF, and VEGF can contribute to neuroprotection.

1. Introduction

In neonates, hypoxic-ischemic brain injury is one of the most common causes of death (Berger and Garnier, 1999) or severe morbidity, with neurodevelopmental sequelae occurring in 30–40% of cases of moderate brain injury, and in more than 90% of cases of severe brain injury (Vannucci and Hagberg, 2004). Although several studies have focused on developing neuroprotective agents and therapies, treatment of hypoxic-ischemic encephalopathy is still mainly supportive. Recently, therapeutic hypothermia has demonstrated a neuroprotective effect in hypoxic--ischemic brain injury, and is now applied clinically, in limited situations (Giesinger et al., 2017).

Research analyzing the ability of stem cells to regenerate tissue after injury has included neuronal tissue. Stem cell therapy is currently in the limelight as a new therapy in brain/nervous system injury, despite a lack of validated applications or transplantation methods (van Velthoven et al., 2010). Neural stem cells (NSCs) are self-renewing cells that can proliferate and differentiate into neurons, astrocytes, and oligodendrocytes; they may play a role in restoring neuronal function (Jin-qiao et al., 2009). NSCs have a certain degree of plasticity, giving rise to endothelial cells that can potentially form capillary networks (Pimentel-Coelho and Mendez-Otero 2010). Most studies in which NSCs were transplanted into a hypoxic-ischemic brain reported a good potential for migration and differentiation. In one study analyzing hypoxic-ischemic rat brains in vivo, NSCs migrated preferentially to the area of injury, and some expressed neuronal markers 14–21 days after transplantation. These results indicate that the environment of the hypoxic-ischemic brain can support NSC migration and neuronal differentiation (Zheng et al., 2006). However, there are a number of problems remaining to be solved for NSC transplanta-

Abbreviations: GLUT1, glucose transporter 1; FGF1, fibroblast growth factor 1; NT3, neurotrophin 3; NT4, neurotrophin 4; HIF, hypoxia inducible factor; NGF, nerve growth factor; GDNF, glial cell-derived neurotrophic factor; VEGF, vascular endothelial growth factor; MCP3, monocyte chemotactic protein 3; GCSF, granulocyte colony-stimulating factor; IGF1, insulin-like growth factor 1; IGF2, insulin-like growth factor 2.

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tion in clinical setting, such as, among others, further brain injury caused by the invasive transplantation procedure, a low survival rate of donor NSCs, limited cell migration, and the risk of tumorigenesis.

To better determine the applicability of NSCs as a therapy for hypoxic-ischemic brain damage, we assessed hypoxic damage in cultured organotypic hippocampal rat brain slices. We also utilized two different growth media, one with and one without stem cell conditioning, to determine which (if any) neurotrophic or growth factors were present that could mediate the therapeutic abilities of NSCs.

2. Results

We induced hypoxic injury in cultured hippocampal tissue slices while using Gahwiler's medium and stem cell-conditioned medium for 14 days. We captured fluorescent images using propidium iodide (PI) and after exposure to NMDA, and compared the injured areas. We divided the tissue slices into four groups: tissues cultured using Gahwiler's medium (G control), hypoxic damage tissues cultured using Gahwiler's medium (G hypoxia), tissues cultured using stem cell-conditioned medium (S control), hypoxic damage tissues cultured using stem cell-conditioned medium (S hypoxia). At day 14 in vitro, the S control group was less damaged than the G control group, and the S hypoxia group was less damaged than the G hypoxia group (Fig. 2).

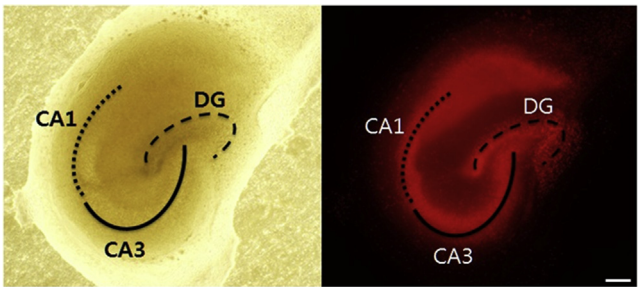

Fig. 1. Location of CA1 (Cornu Ammonis 1), CA3 (Cornu Ammonis 3), DG (Dentate Gyrus) in the cultured slices (scale bar: 200 μm).

As presented in Fig. 3, we also quantified the injured area according to the CA1, CA3 and DG subregions. In the CA1 region, the G control and S control groups were not significantly different from each other ($p = 0.095$), but in the hypoxia groups, with the S group ratio (ratio: see method section, 4.4 Image analysis) being significantly smaller than the G group ratio ($p < 0.05$). While the G hypoxia group ratio was higher (i.e., showing more hypoxic damage) than the G control group, but not significant ($p = 0.166$), the S hypoxia and S control groups were not significantly different from each other ($p = 1.0$). The S hypoxia group was significantly less damaged than the G hypoxia group ($p < 0.05$). In the CA3 subregion, the G hypoxia group ratio was not significantly higher than that of the G control group ($p = 0.462$), and the S hypoxia and S control groups were not significantly different from each other ($p = 0.983$). There were significant differences between the G hypoxia and the S hypoxia groups ($p < 0.05$) with the S group being significantly less damaged than its corresponding G group. In the DG, there were significant differences between the G control and the S control groups ($p < 0.05$); however, the G hypoxia group was not significantly different from the G control group ($p = 1.0$), nor was the S hypoxia group significantly different from the S control group ($p = 0.788$) (Fig. 3).

Neurons were immunostained with mixed neurofilament (NF-mix). The expression of NF-mix was increased in the S hypoxia group, compared to the G hypoxia group, suggesting a neuroprotective effect of secreted factors in the S-medium (Fig. 4).

Gene expression of neurotrophic factors/growth factors in F3 (human) NSCs was studied using RT-PCR. The results demonstrated that F3 NSCs can express the NGF, BDNF, NT-3, GDNF, CNTF, bFGF, and VEGF gene products (Fig. 5). To examine neuroprotective

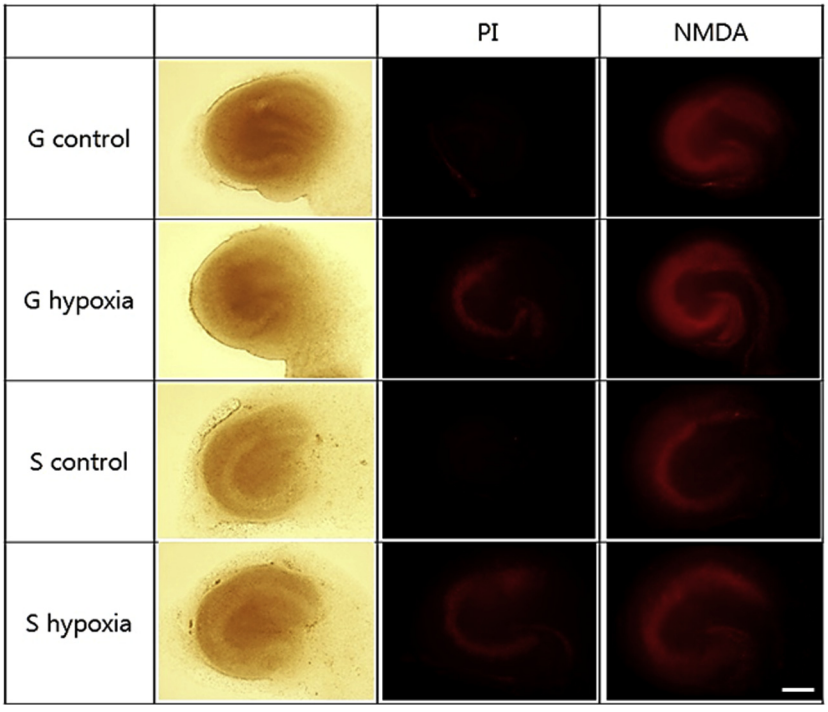

Fig. 2. Images of hippocampal slices at 14 days in vitro (scale bar: 400 μm): G control, Gahwiler's medium before hypoxic injury; G hypoxia, Gahwiler's medium after hypoxic injury; S control, stem cell-conditioned medium before hypoxic injury; S hypoxia, stem cell-conditioned medium after hypoxic injury.