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Research report

Effect of enriched environment on angiogenesis and neurological functions in rats with focal cerebral ischemia

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ABSTRACT

The purpose of this study was to investigate the effect of enriched environment (EE) on cerebral angiogenesis after ischemia-reperfusion injury. Middle cerebral artery occlusion (MCAO) followed by reperfusion was performed in rats to set up an animal model of ischemia-reperfusion injury. In a set of behavioral tests, we demonstrated that the animals in the IEE (ischemia + enriched environment) group exhibited significantly improved neurological functions compared to those in the standard housing condition group. In consistent with the functional tests, smaller infarction volumes were observed in the animals of IEE group. Laser scanning confocal microscopy and 3D quantitative analysis of cerebral microvessels revealed that EE treatment increased the total vessel surface area and number of branch point in the ischemic boundary zone. IgG extraction assay showed that the blood brain barrier (BBB) leakage in the ischemic brain was attenuated after EE treatment. EE treatment also enhanced endothelial cells (ECs) proliferation and increased the expression levels of VEGF and its receptor Flk-1 after ischemia-reperfusion injury. Analyses of Spearman's correlation coefficients indicated a correlation of mNSS scores with enhanced cerebral angiogenesis. Together, the results suggest that EE treatment-induced cerebral angiogenesis may contribute to the improved neurological outcome of stroke animals after ischemia-reperfusion injury.

1. Introduction

Stroke is the leading cause of disability worldwide which is associated with serious neurological impairment and persistent physical deficits (Go et al., 2014). Although some progress in rehabilitation has been made to improve the survivors' quality of life, it still imposes great strain to the patients, families and societies. Thus, studies aiming to elaborate the pathophysiological background of the stroke recovery process are needed to explore novel therapeutical strategies. Ischemic stroke is due to occlusion of a cerebral blood vessel. The severe reduction of blood flow leads to a lack of oxygen and nutrient transportation, which ultimately ends up with tissue hypoxia and cells death. Therefore, the restoration of cerebral circulation is a potential mechanism to compensate for the detrimental effects in the acute and recovery stage of stroke.

Angiogenesis is defined as the formation of new blood vessels by sprouting of endothelial cells from pre-existing vessels, which is the main style of new blood vessels formation under pathological condition in adult. It can be interpreted as a natural defense mechanism helping to restore oxygen and nutrient supply to the ischemic brain tissue (Risau, 1997; Beck and Plate, 2009). A lot of evidences have shown that vascular-remodeling occurs after stroke (Beck and Plate, 2009; Li et al., 2015; Zhao et al., 2015). Human studies have suggested that active angiogenesis takes place at 3–4 days after ischemic insult, and stroke patients with higher microvessel density in the ischemic border zone are coupled with longer survival time and lower morbidity (Krupinski et al., 1994). Therefore, angiogenesis might be a promising mechanism underlying post-stroke recovery (Ergul et al., 2012). During the process of angiogenesis, endothelial cells (ECs) degrade the underlying basement membrane, migrate into neighboring tissue, proliferate, and

Abbreviations: EE, enriched environment; MCAO, middle cerebral artery occlusion; VEGF, vascular endothelial growth factor; ECs, endothelial cells; IgG, Immunoglulin G; BBB, blood brain barrier; LSCM, laser scanning confocal microscopy; IEE, ischemia + enriched environment; ISC, ischemia + standard conditioning; SSC, sham + standard conditioning; mNSS, modified Neurological Severity Score

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assemble into tubes which connected to each other and then blood flow is established (Marti et al., 2000). The formation of a mature vasculature demands not only cells proliferation but also soluble factors to modulate cell-cell and cell-matrix interactions (Risau, 1996). Vascular endothelial growth factor (VEGF) is the most prominent members of angiogenic growth factors, which play a critical role for endothelial cell growth and differentiation by binding to its receptors, Flt-1 and Flk-1 (Argandoña et al., 2012; Takahashi and Shibuya, 2005). Hermann and Zechariah (2009) have reported that the expression of VEGF is associated with an increase in vascular density in the ischemic penumbra, suggesting that VEGF plays an important role in angiogenesis following brain ischemia.

An enriched environment is a rehabilitation strategy for rodents, in which multiple animals are housed together in a large cage equipped with different toys and enhanced novelty and complexity compared to standard conditions (Hepp, 1947; Madinier et al., 2014). Mounting evidences have suggested that exposure to EE after brain injury may lead to neuroprotective effects and improved functional outcomes by inducing neurogenesis, increasing dendritic branching and spine density, promoting trophic factors production and changes in gene expression (He et al., 2010; Hu et al., 2010; Jung and Herms, 2014; Yuan et al., 2012; Zhang, et al., 2015). Prior exposure to EE is also reported to increase ischemic tolerance of the brain and improve motor function (Xie et al., 2013). However the detailed mechanisms underlying the functional recovery processes are still poorly understood.

We hypothesized that EE treatment-induced cerebral angiogenesis might lead to a functional improvement after ischemia-reperfusion injury. We therefore investigated whether EE enhanced post-stroke angiogenesis, and if so, whether these changes facilitate functional recovery of stroke animals. We performed immunocytochemical experiments to determine the expression of VEGF, Flk-1, IgG extraction, and the co-expression of CD31/Ki67. We tested the angiogenesis in the ischemic boundary zone of cortex using laser scanning confocal microscopy (LSCM). We also examined the effects of EE treatment on the neurological deficits and infarct volume at 14 days after MCAO.

2. Results

2.1. EE treatment improves the functional recovery in stroke animals

In order to examine the effect of EE treatment on the neurological function of stroke animals, a set of behavior tests, including the Modified Neurological Severity Score (mNSS) and rota-rod test and beam walking test (BWT), were carried out at 3, 7, 14 days after MCAO by an investigator who was unaware of the experimental groups. As shown in Fig. 1, rats in sham + standard conditioning (SSC) group had mild neurological symptoms but fully recovered 3 days after surgery. There was no difference between ischemia + enriched environment (IEE) group and ischemia + standard conditioning (ISC) group at day 3. However, by the time of day 7 and 14, the mNSS scores in IEE group was significantly lower than that in ISC group. The same trend was observed in the rota-rod test. The ischemic rats in both IEE and ISC groups continued to show an increase in the time they stayed on the rod during the two weeks following stroke. Treatment with EE significantly prolonged the time to fall from the rod at day 7 and 14 compared to the ISC group. In BWT, no significant difference was seen between ISC and IEE groups on day 3 and day 7, but the rats in IEE group had significantly higher scores on day 14 compared to ISC group. These results indicate that animals in IEE group had improved functional outcome.

2.2. EE treatment reduces the infarct volume after cerebral ischemia-reperfusion injury

As the improved functional outcome would be resulted from the reduced brain damage, we tested the effect of EE on brain infarct volume by performing Nissl staining at 14 days after MCAO (Fig. 2). As shown in Fig. 2, no lesion was found in SSC group. Infarct volume was significantly larger in ISC group compared with IEE group, which was consistent with the observations in the preceding neurobehavioral tests.

2.3. Effect of EE treatment on neovascularization in ischemic boundary zone

To determine whether EE-induced neuroprotection is mediated at least in part by angiogenesis in the ischemic brain region, laser scanning confocal microscopy (LSCM) followed by Three-dimensional (3D) quantitative analysis of the cerebral microvessels was performed. Fig. 3A, D, and G show the images of the reconstructed 3D cerebral microvessels in the penumbra. The irregular pattern of vessel tortuosity suggests that there were newly generated vessels in both ISC and IEE group (Fig. 3D and G). Different colors in Fig. 3B, E and H represented individual vessels that were not connected to each other. Green and red colors in Fig. 3C, F, and I code for diameter of blood vessels ≤7.5 μm (green) and >7.5 μm (red). The capillaries in the ischemic boundary zone of ISC group exhibited a significantly smaller diameter compared with SSC group, while EE treatment increased vascular diameter after stroke compared with ISC group (Table 1, Fig. 3). The number of vascular branch points was higher in the IEE group compared with ISC group and SSC group. EE treatment also significantly increased the total surface area of microvessels compared to other groups (Table 1, Fig. 3). These data suggest that EE treatment promotes angiogenesis after ischemia-reperfusion injury.

2.4. EE treatment attenuates BBB leakage after ischemia-reperfusion injury

To test the effect of EE treatment on the integrity of BBB after ischemia-reperfusion injury, we measured the leakage of serum IgGs by

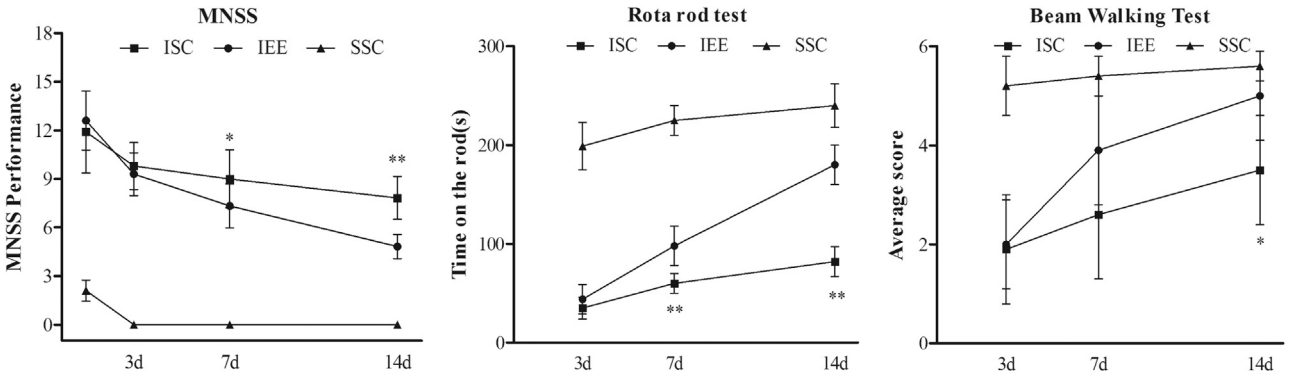

Fig. 1. Neural function outcomes. The data were presented as mean ± SD. $^{*}p < 0.05$ compared to IEE group; $^{**}p < 0.01$ compared to IEE group.