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INHIBITION OF INTERLEUKIN-6 ABOLISHES THE PROMOTING EFFECTS OF PAIR HOUSING ON POST-STROKE NEUROGENESIS

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Abstract—Interleukin-6 (IL-6) has been shown to promote post-stroke angiogenesis and long-term functional recovery; however, whether IL-6 could promote post-stroke neurogenesis remains unclear. This study aims to investigate the effects of IL-6 on neurogenesis after ischemic stroke. We also investigated whether pair housing (PH) could improve the experimental stroke outcome through IL-6. Transient middle cerebral artery occlusion (tMCAO) was induced in mice treated with recombinant IL-6 (rIL-6) or anti-IL-6 neutralizing antibodies (anti-IL-6 mAbs). Another set of mice were pair-housed (PH; male and ovariectomized female) for 2 weeks, subjected to tMCAO and then assigned to a housing condition (isolated or PH). Pair-housed mice were treated with anti-IL-6 mAbs. Behavioral assessments were made 3 days before tMCAO and after 28 days of reperfusion. Neural progenitor cells (NPCs) isolated from ipsilateral subventricular zone (SVZ) at 14 days post-ischemia were treated with rIL-6 plus soluble IL-6 receptor (sIL-6R). The effects of IL-6 on the proliferation and differentiation of NPCs were examined *in vivo* and *in vitro*. The role and mechanism of IL-6 in PH-mediated enhancement of NPC proliferation and functional recovery were investigated *in vivo*. We found that anti-IL-6 mAbs significantly reduced the proliferation and neuronal differentiation of NPCs in the ipsilateral SVZ, as well as functional recovery; whereas rIL-6 conferred the opposite effects. PH significantly promoted NPC proliferation and functional recovery compared with socially isolated cohorts; blockade of IL-6 with anti-IL-6 mAbs prevented this promoting effect. In conclusion, our results suggest that IL-6 is an important mediator of social interaction on neurogenesis and long-term functional recovery after ischemic stroke.

Key words: IL-6, ischemic stroke, middle cerebral artery occlusion, neural progenitor cells, pair housing.

INTRODUCTION

Interleukin-6 (IL-6), as well as ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF), belong to the family of glycoprotein 130 (gp130)-activating cytokines. IL-6 acts on target cells through a membrane glycoprotein, gp130, which associates with IL-6 receptor (Heinrich et al., 2003). Alternatively, IL-6 can also signal through a soluble IL-6 receptor. Depending on the cellular context, IL-6 may exert an array of diverse and competing effects including anti-apoptotic, pro-proliferative and differentiation-inducing effects. It has been shown that IL-6 could promote neuronal differentiation of neural stem/progenitor cells (NSPCs) dissociated from normal adult mice (Barkho et al., 2006). Under physiological conditions, adult IL-6 knockout mice exhibit significantly lower neural progenitor cell (NPC) survival and proliferation in the dentate gyrus (DG) and subventricular zone (SVZ) (Bowen et al., 2011). A recent study showed that IL-6 produced locally by resident brain cells promotes angiogenesis in the delayed phases of stroke recovery (Gertz et al., 2012). However, it is unclear whether IL-6 could affect post-stroke neurogenesis, which has been shown to be coupled with angiogenesis in brain tissue repair and remodeling after stroke (Kojima et al., 2010).

Research suggests that stroke survivors often experience social isolation. Social interaction could improve quality of life and decreases mortality after stroke (Boden-Albala et al., 2005; Hinojosa et al., 2011). In order to discover the biological mechanisms underlying the benefits of social interaction, development of preclinical animal studies are required. Pair housing (PH) of mice could well reproduce social interaction in animal studies, and social companionship provided by PH encourages social interaction (Craft et al., 2005; Venna et al., 2014). In recent years, increasing evidences have

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Abbreviations: BrdU, 5-Bromo-2′-deoxyuridine; CCA, common carotid artery; CNTF, ciliary neurotrophic factor; DCX, doublecortin; DG, dentate gyrus; EBST, elevated body swing test; ECA, external carotid artery; gp130, glycoprotein 130; i.c.v., intracerebroventricle; IL-6, Interleukin-6; LIF, leukemia inhibitory factor; MCAO, middle cerebral artery occlusion; NPCs, neural progenitor cells; PH, pair housing; rIL-6, recombinant IL-6; RT-PCR, real-time PCR; SBDP145, aII-spectrin breakdown products of 145 kDa; sIL-6R, soluble forms of the IL-6 receptor; SVZ, subventricular zone; tMCAO, Transient middle cerebral artery occlusion.

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shown that PH could decrease infarct size and brain atrophy, and increase neurogenesis and long-term functional recovery after ischemic stroke (Craft et al., 2005; Karelina et al., 2009; O'Keefe et al., 2014; Venna et al., 2014; Verma et al., 2014). However, the underlying mechanisms are poorly understood. A previous study showed that PH increases central levels of IL-6 in the acute phase of stroke, and treatment with the IL-6 neutralizing antibody increases infarct volume and eliminates the effect of PH on infarct size (Karelina et al., 2009). However, it is still unclear whether PH could increase central expression of IL-6 and improve long-term functional recovery through IL-6 in the later phases of stroke recovery.

Here we initially investigated the effects of IL-6 on the post-stroke neurogenesis using experimental stroke models and primary NPC cultures obtained from the ipsilateral SVZ at 14 days post-ischemia. Our results indicated that IL-6 played important roles in the maintenance and augment of ischemia-induced proliferation and neuronal differentiation of SVZ NPCs, as well as long-term functional recovery. In addition, we went on to investigate the role of IL-6 in PH-mediated effects on the proliferation of NPCs and long-term functional recovery. We found that PH increased the expression of IL-6 in the ipsilateral hemisphere through inhibition of calpain activity during stroke recovery. PH improved the proliferation of SVZ NPCs and long-term functional recovery; anti-IL-6 neutralizing antibodies abrogated the effects. Our results suggest that IL-6 is essential for the promoting effects of social interaction on the neurogenesis and long-term outcome after ischemic stroke.

EXPERIMENTAL PROCEDURES

Animals

Male C57BL/6 mice (8–10 weeks old, 23–25 g) were purchased from Wuhan University Laboratory Animal Center. All experimental protocols and animal handling procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996 and the experimental protocols were approved by the committee of experimental animals of Tongji Medical College.

Middle cerebral artery occlusion (MCAO) model of focal ischemia

The animals were maintained at constant room temperature (23.0 ± 1.0 °C), humidity 55–65% and 12-h light–dark cycle. Anesthesia was induced with ketamine (100 mg/kg, i.p.) and xylazine (8 mg/kg, i.p.). Focal cerebral ischemia was induced by transient occlusion of the right middle cerebral artery (tMCAO) with a 6–0 silicone-coated nylon monofilament, as previously described (Hata et al., 1998). Briefly, under the operating microscope, the right common carotid artery (CCA), the right external carotid artery (ECA), and the right internal carotid artery (ICA) were isolated and a 6–0 suture was tied at the origin of the ECA and at the distal end of the ECA. The right CCA and ICA were temporarily occluded. The silicon-coated nylon suture was introduced into the ECA and pushed up the ICA until resistance was felt and the filament was inserted about 9 to 10 mm from the carotid bifurcation, effectively blocking the middle cerebral artery (MCA). The diameter of the tip of coated suture was considered acceptable between 180 and 220 μm. The suture remained inserted for 60 min, after which it was removed and the ECA was permanently tied. In the sham controls, the arteries were visualized but not disturbed. Occlusion was confirmed by laser-Doppler flowmeter (Periflux system 5000, PERIMED, Stockholm, Sweden) with a probe placed on thinned skull over the lateral parietal cortex (Zhang et al., 2014). Body temperature was maintained at 37 ± 0.5 °C with a feed-back temperature control unit until the mice had recovered from surgery. Subcutaneous normal saline (0.9%) was administered daily, adjusting the volume according to the animal's weight loss. After mice had recovered from surgery, an 18-point scoring system was used to evaluate the sensorimotor deficits (Zhang et al., 2014). The number of animals used for each experimental group is provided in the figure legends and summarized in Table 1.

In experiment 1, C57BL/6 mice were subject to sham or 60-min ischemia followed by 1, 3, 6, 14, 21, 28, and 42 days of reperfusion to measure the expression patterns of IL-6 (Fig. 1A).

In experiment 2, C57BL/6 mice were randomly divided into five groups: (1) Cerebral I/R group (MCAO) (60 min ischemia followed by 28 days reperfusion); (2–3) IL-6 neutralizing antibodies (anti-IL-6 mAbs) treatment group: Intracerebroventricular cannulation was performed according to previously established protocols (Karelina et al., 2009). Mice received intracerebroventricularly (i.c.v.) either anti-IL-6 mAbs (10 ng in 2 μl aCSF) (R&D Systems, Minneapolis, MN, USA) or 2-μl aCSF. This dose has been used successfully to neutralize IL-6 signaling in mice (Meagher et al., 2007); (4–5) recombinant mouse-IL-6 (rIL-6) treatment group: A sterile 26-G Hamilton microsyringe (80330; Hamilton Company, Reno, NV, USA) was used to intranasally administer 2-μl drops of rIL-6 (R&D Systems, Minneapolis, MN) diluted in PBS (0.01 μg/μl) or its vehicle (PBS) to alternating nostrils with a 2-min interval between applications. Drops were placed at the opening of the nostril, allowing the mice to snort each drop into the nasal cavity. A total of 10 μl of dose solution, containing 0.1 μg rIL-6 was delivered over a course of 5 min. The injection of anti-IL-6 mAbs (or aCSF) or rIL-6 (or vehicle) was repeated every 24 h for 2 week starting at 14dpi (Fig. 1B).

Experiment 3 was designed to test the role of IL-6 in mediating the effects of social interaction on stroke outcome. C57BL/6 mice were housed either individually (socially isolated) or with an ovariectomized female (PH) for a period of 2 weeks before surgery and throughout the reperfusion period. Anti-IL-6 mAbs (10 ng in 2 μl aCSF) or 2-μl aCSF was administrated i.c.v. to pair-housed mice for 2 weeks starting at 14dpi. Calpain inhibitor calpeptin (10 μg dissolved in 2 μl 10% DMSO;