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Noggin improves ischemic brain tissue repair and promotes alternative activation of microglia in mice

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ABSTRACT

We previously reported that bone morphogenetic proteins (BMPs) and their endogenous antagonist noggin are expressed in the brain weeks after an ischemic insult. Here, to define their roles in ischemic brain tissue repair and remodeling, we infused recombinant BMP7 or noggin into the ipsilateral ventricle of mice for 2 weeks starting 2 weeks after transient middle cerebral artery occlusion (MCAO). Four weeks after MCAO, we measured ischemic brain volume, functional recovery, and molecules related to neurogenesis and angiogenesis such as synaptophysin, GAP-43, and VEGF. Noggin-treated mice but not BMP7-treated mice showed preserved ipsilateral brain volume and reduced neurological deficits compared with artificial cerebrospinal fluids (aCSF)-treated mice. Noggin treatment also decreased glial scar thickness, increased levels of GAP-43 and VEGF protein, and increased the number of Iba1-positive activated microglia in the ipsilateral brain. Furthermore, noggin treatment decreased M1 markers (IL-1β, TNF-α, IL-12, CCL2 and CD86) and increased M2 markers (IL-1ra, IL-10, arginase 1, CD206 and Ym1) of activated microglia, suggesting a shift from M1 to M2 phenotypes. These results suggest that noggin improves functional recovery from ischemic stroke and enhances alternatively activated microglia, thereby promoting tissue repair and remodeling.

1. Introduction

Ischemic stroke, a leading cause of mortality worldwide, results in mild to severe functional disabilities unless spontaneous or thrombolytic reperfusion occurs within hours. Patients who survived ischemic stroke show variable degrees of recovery over time, suggesting that endogenous neuroreparative processes such as neurogenesis, axonal sprouting, gliogenesis, and angiogenesis occur in the diseased brain (Cramer, 2008a; Gutierrez-Fernandez et al., 2012). Because only a minority of patients receive thrombolytic therapy due to its narrow therapeutic window, facilitating brain repair processes by neurorestorative therapy in the weeks after ischemic stroke is an approach that deserves further investigation (Cramer, 2008b).

Functional recovery from stroke depends on a balance between factors that impede tissue repair and factors that promote brain plasticity and repair (Popa-Wagner et al., 2011). Glial scars, which are primarily composed of reactive astrocytes, protect intact tissue by demarcating the ischemic area during early phases of ischemic stroke but impede tissue repair in the long term (Anderson et al., 2003; Roitbak and Sykova, 1999). For example, accelerated glial scar formation after an ischemic insult is associated with poorer functional recovery in aged animals compared with young animals (Badan et al., 2003). On the other hand, bone marrow stromal cell administration, hepatocyte growth factor gene transfer, or intraventricular infusion of erythropoietin after ischemic stroke reduces scar wall thickness, and this is related to improved behavioral outcome and brain repair processes such as axonal regeneration and angiogenesis (Li et al., 2005; Reitmeir et al., 2011; Shimamura et al., 2006). Therefore, identifying and defining the roles of molecules involved in scar formation and resolution in the ischemic brain will lead to the development of novel neurorestorative therapies.

Bone morphogenetic proteins (BMPs) and their endogenous antagonist noggin are involved in repair processes after spinal cord injury and brain diseases. Increases in BMPs at the site of demyelinating lesions of spinal cord and corpus callosum are associated with astrogliosis and inhibition of mature oligodendrocyte

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differentiation of oligodendrocyte precursor cells (Dummula et al., 2011; Fuller et al., 2007; Wang et al., 2011). Also, BMPs promote astrocyte differentiation from postnatal cortical oligodendroglial-astroglial progenitor cells and neural stem cells while suppressing the production of neurons and oligodendrocytes, suggesting astroglial inductive action of BMPs (Mabie et al., 1997; Xiao et al., 2010). In patients with Alzheimer's disease (AD) and a mouse model of AD, increased BMP levels correlate with reduced hippocampal neurogenesis (Bonaguidi et al., 2008; Crews et al., 2010). By contrast, noggin infusion during demyelination reduces astrocyte numbers but increases mature oligodendrocytes and remyelination by blocking phosphorylation of Sma and Mad Related Family (Smad) proteins downstream of BMP signaling (Cate et al., 2010; Sabo et al., 2011).

Increases in BMPs and noggin have been reported in the ischemic brain (Chang et al., 2003; Lei et al., 2012; Shin et al., 2012), but their roles in repair processes during later phases after ischemic stroke are not clearly defined. Reports that BMPs, such as BMP7, are involved in the improvement of recovery after ischemic insult (Chang et al., 2003; Chou et al., 2006) raise the possibility that the actions of BMPs might differ in the ischemic brain. Our previous study reporting temporal changes in BMPs and noggin expression provides clues for their neurorestorative actions after ischemic insult. BMPs and noggin are differentially expressed across the first 4 weeks after transient ischemic stroke, and their expression is related to astroglial scar formation (Shin et al., 2012). Increased BMP2 and BMP7 expression were observed starting 1 week and lasting 4 weeks after ischemic stroke, and their levels correlated with astrogliosis. Compared with BMPs, noggin expression was relatively weak but significantly increased 2 weeks and further increased 4 weeks after ischemic stroke. We also showed that expression of BMPs and noggin are associated with glial fibrillary acidic protein (GFAP)-positive astrocytes and ionized calcium-binding adapter molecule 1(Iba1)-positive microglia, respectively, in the ischemic brain. These findings suggest that BMP7 and noggin have different roles in repair processes during chronic phases after ischemic stroke (Shin et al., 2012).

Here, to define the roles of BMP7 and noggin in brain repair after ischemic stroke, we infused each recombinant protein into the ipsilateral ventricle of ischemic brains to boost protein levels 1–2 weeks after insults, as increased BMP7 and noggin protein levels were previously observed together with astrogliosis during this time periods. Also, as early glial scar formation has a crucial role in sealing the lesion site and preserving spared tissue during the acute phase after insults (Rolls et al., 2009), the effects of recombinant protein infusion were tested during subacute and chronic stages. Ischemic brain atrophy, neurological deficits, and molecules related to brain repair processes were examined in mice-treated with recombinant proteins 4 weeks after ischemic stroke.

2. Material and methods

2.1. Animals

Male C57BL/6 mice, aged 10–11 weeks, were used in the experiments (Orient Bio Inc., Seongnam, Republic of Korea). All procedures were approved by the Institutional Animal Care and Use Committee at the Medical School of Ewha Womans University and conformed to international guidelines for the ethical use of experimental animals. Mice were acclimatized to the animal colony, which was kept under a 12-h light/dark cycle at 22 ± 2 °C for 2 weeks prior to the experiments. The number of animals used was minimized to reduce animal suffering. Ischemic stroke was induced in 145 mice, 19 mice of which were excluded according to the criteria of changes in cerebral blood flow (CBF) during surgical procedures (see below). Thirty-four mice died due to brain swelling within 1 week of ischemic stroke, resulting in 92 mice that survived 2 weeks after surgery. For determination of the dose and duration of recombinant protein infusion, 24 surviving mice were randomly assigned to aCSF, noggin or and BMP7 groups (0.5 or 1 μg protein per day for 2 or 3 weeks, n = 3–4 per group). To measure ischemic brain volume, 46 surviving mice were randomly assigned to aCSF, noggin or BMP7 groups (n = 15–16 per group). Twenty-two surviving mice were randomly assigned to aCSF and noggin groups for western blotting (n = 6 per group) and immunofluorescence staining (n = 5 per group). Naïve control mice were also used for western blotting and immunofluorescence staining (n = 5 in each). Therefore, the total number of mice included in our analysis was 102.

2.2. Transient middle cerebral artery occlusion (MCAO)

Procedures for transient MCAO were previously described (Shin et al., 2009). Briefly, mice were anesthetized with isoflurane, and a fiber optic probe was attached to the right parietal bone (2 mm posterior and 5 mm lateral to bregma) and connected to a laser-Doppler flowmeter (Periflux System 5010, Perimed, Sweden). CBF was continuously recorded during MCAO and reperfusion periods with a computer-based data acquisition system (Perisoft, Perimed, Sweden). A 6–0 silicon-coated black monofilament surgical suture (Doccol Cooperation, Redlands, CA, USA) was inserted into the exposed right external carotid artery, advanced into the internal carotid artery, and wedged into the circle of Willis to obstruct the origin of the MCA. The filament was left in place for 30 min and then withdrawn to re-establish CBF. Only animals that exhibited a greater than 85% reduction in CBF during MCAO that recovered by more than 80% after 10 min of reperfusion were included in the study. Rectal temperature was maintained at 37.0 ± 0.5 °C with a thermostatically controlled heating pad during surgery and recovery until mice regained consciousness.

2.3. Intraventricular infusion

Recombinant human noggin (R&D Systems, Minneapolis, MN, USA) and human BMP7 (Biovision, Inc., Militas, CA, USA) were dissolved in artificial cerebrospinal fluid (aCSF; R&D Systems). Each protein or aCSF was delivered to the ipsilateral lateral ventricle by ALZET osmotic pumps (DURECT Corporation, Cupertino, CA, USA) according to the manufacturer's instructions. A cannula (ALZET Brain infusion kit 3, DURECT Corporation) connected to the osmotic pumps was implanted 0.5 mm anterior to bregma, 1 mm lateral from the midline, and 3 mm below the surface of the skull. Mice received 0.5 or 1 μg of protein per day for 2 weeks starting 2 weeks after MCAO or for 3 weeks starting 1 week after MCAO. These test doses were based on previous studies (Cate et al., 2010; Colak et al., 2008).

2.4. Behavioral tests

Neurological scores and performance in the wire suspension test were examined prior to and weekly after MCAO. Neurological scores were based on a four-point graded scoring system (Li et al., 2004): 0 = no deficit, 1 = forelimb weakness and torso turning to the ipsilateral side when held by the tail, 2 = circling to the affected side, 3 = unable to bear weight on the affected side, and 4 = no spontaneous locomotor activity or barrel rolling. The wire suspension test was modified from a previous study (Takahashi et al., 2009). A mouse was placed on a wire (60 cm in length and 2 mm in diameter, elevated up to 60 cm from a surface) at a point midway between two support posts for 30 s. Performance was evaluated using the following scores: 0 = fell off the wire, 1 = hung