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EFFECTS OF STROMAL CELL-DERIVED FACTOR 1α DELIVERED AT DIFFERENT PHASES OF TRANSIENT FOCAL ISCHEMIA IN RATS

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Abstract—Endogenous stromal cell-derived factor 1α (SDF1α) has been implicated in postischemic tissue repair, suggesting SDF1α as a potential therapeutic molecule to treat stroke patients. In spite of its potential, no data are available regarding the short- and long-term effects of SDF1α when it is delivered at different phases of stroke. In our study, adenovirus expressing SDF1α gene (AV-SDF1α) was introduced into the boundary of the infarcted area either 3 days before or 1 week after ischemia, and behavioral performance was measured over 5 weeks. Immediate behavioral and structural amelioration was evident when AV-SDF1α was injected 3 days before ischemia, which might be the result of SDF1α-mediated neuroprotection as supported by the TUNEL staining and Western blot analysis of active caspase-3. In addition, increase in neurogenesis, neuroblast migration, and neural differentiation was also apparent in the AV-SDF1α-injected brain, which contributed to further amelioration at later time points ("delayed response"). On the contrary, when AV-SDF1α was introduced 1 week post-ischemia (in the subacute phase), significant behavioral recovery became apparent beginning 5 weeks after viral delivery. Taken together, the therapeutic efficacy of SDF1α varied considerably depending on when SDF1α overexpression was initiated; initiating SDF1α overexpression before ischemia exerted both immediate and delayed beneficial effects, whereas initiating overexpression in the subacute phase exerted only a delayed response.

Key words: ischemic stroke, neurogenesis, neuroprotection, stromal cell-derived factor 1.

Ischemic stroke is the leading cause of long-term disability in developed countries. Surprisingly, however, therapeutic options to treat this devastating disease are rather limited. Current strategies for the development of therapeutics for stroke include a wide range of approaches, such as neuroprotective therapy to prevent further neuronal death primarily in the penumbra area and regenerative strategy to replace dead neurons and glial cells with new cells (Zaleska et al., 2009; Moskowitz et al., 2010).

Regenerative therapy induces functional recovery by promoting neural differentiation either from the grafted cells (Lee et al., 2008) or from the endogenous neural stem cells (Arvidsson et al., 2002), followed by functional integration of the newborn neurons into the existing neural circuits. Because active neurogenesis was shown to be evident in the brain of human (Jin et al., 2006) and animal models (Arvidsson et al., 2002; Thored et al., 2006) after ischemic stroke, reconstructing the damaged brain area with newborn neural cells migrated from the neurogenic subventricular zone (SVZ) might be an attractive option.

Stromal cell-derived factor 1 (SDF1) is a cytokine that belongs to the CXC chemokine family that is officially designated chemokine (C-X-C motif) ligand 12 (CXCL12). Two forms of SDF1—SDF1α/CXCL12a and SDF1β/CXCL12b—are produced by alternative splicing of a single gene (Baggiolini et al., 1997). The receptor for SDF1 is CXCR4, and the binding of SDF1 to CXCR4 is involved in the modulation of peripheral immune cells and the homing of bone marrow stem cells (Aiuti et al., 1997). Recently, the SDF1/CXCR4 axis has been shown to modulate neuronal activity (Guyon et al., 2005) and promote neuronal survival (Wu et al., 2009). SDF1 has been implicated in the migration of endogenous neural stem cells to the injury site after stroke (Imitola et al., 2004; Robin et al., 2006; Thored et al., 2006; Kokovay et al., 2010), and blocking CXCR4 decreases the migration of neural stem cells to the damaged area in both *in vitro* (Robin et al., 2006) and *in vivo* (Thored et al., 2006) models. Consistent with these observations, after the onset of stroke, SDF1 expression increases substantially near the infarct area and lasts for several weeks thereafter (Hill et al., 2004), thereby contributing—at least in part—to poststroke recovery.

Because of its role in recovery after ischemic brain damage, SDF1 is a potential candidate for therapeutic regimens for stroke patients. In support of this notion, delivering exogenous SDF1 into the rat brain 30 min after ischemia (i.e., in the acute phase) rescued both the damaged tissue and behavioral performance to some degree when analyzed at 3 days after ischemia (Shyu et al., 2008). However, no information is available regarding the effect of exogenous SDF1 when delivered at other phases of ischemic injury. Furthermore, long-term effects of the SDF1 delivery are yet to be examined. In this study, we examined

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Abbreviations: AV-Control, a recombinant adenoviral vector without SDF1α cDNA; AV-SDF1α, a recombinant adenoviral vector containing a myc-tagged SDF1α cDNA; BrdU, 5-bromo-2-deoxyuridine; CMV, cytomegalovirus; CXCL12, chemokine(C-X-C motif) ligand 12; DCX, doublecortin; ELISA, enzyme-linked immunosorbent assay; GFAP, glial fibrillary acid protein; GFP, green fluorescent protein; HPF, high-power field; HRP, horseradish peroxidase; MCAO, middle cerebral artery occlusion; mNSS, modified neurological severity score; MOI, multiplicity of infection; NSC, neural stem cell; PBS, phosphate-buffered saline; PSA-NCAM, polysialic acid neural cell adhesion molecule; NeuN, neuronal nuclei; PDMS, periventricular dorsomedial striatum; SDF1, stromal cell-derived factor 1; SVZ, subventricular zone; TBS, Tris-buffered saline.

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the outcome of introducing exogenous SDF1 into the rat brain 3 days before (in the pre-ischemic phase) or 1 week after (in the subacute phase) the onset of ischemia and evaluated the effects over 5 weeks. Both immediate beneficial effects and gradual delayed improvement were observed when the SDFI gene was delivered before ischemic injury, whereas only the delayed beneficial effects were detected when the gene was delivered in the subacute phase of stroke.

EXPERIMENTAL PROCEDURES

Generation of the middle cerebral artery occlusion (MCAO) model

This study was approved by the CHA University Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering and to reduce the number of animals used. Adult male Sprague–Dawley rats weighing 240–270 g (Orient Bio, Inc., Seongnam, Korea) underwent transient middle cerebral artery occlusion (MCAO) surgery (Longa et al., 1989). In brief, the common carotid artery, internal carotid artery, and external carotid artery of rats were exposed by a cervical incision followed by cauterization of the superior thyroid and occipital arteries. A silicon-coated 4.0 monofilament nylon suture (length 23 mm; diameter 0.25–0.30 mm) (Ethicon, Somerville, NJ, USA) was introduced into the lumen of the external carotid artery and gently advanced through the internal carotid artery until the origin of the middle cerebral artery was occluded. The ipsilateral common, internal, and external carotid arteries were ligated to temporarily interrupt the blood flow. Withdrawing the suture from the external carotid artery 90 min after the MCAO-induced reperfusion. The sham-operated group was subjected to the same surgical procedure except for the insertion of the suture into the artery.

Adenovirus production

First, the myc-tagged SDF1α cDNA was subcloned into the pAdTrack-cytomegalovirus (CMV) plasmid. The resulting plasmid was linearized with *Pme*I, subjected to homologous recombination with pAdEasy1 in the *E. coli* stain BJ5183, and then transfected into HEK293T cells using Fugene HD (Roche Applied Science, Mannheim, Germany) for viral packaging. After 7–10 days, adenovirus was harvested from the HEK293T cells by three freeze/thaw cycles and then purified by cesium chloride ultracentrifugation. The viral stocks were titrated by infecting HeLa cells, followed by flow cytometric analysis of green fluorescent protein (GFP) expression.

Transduction of adenovirus into the rat brain

Animals were randomly assigned to AV-Control or AV-SDF1α. The rats were anesthetized using an i.p. injection of ketamine (50 mg/kg) and xylazine (5 mg/kg) and placed in a stereotaxic frame (Stoelting Co., Wood Dale, IL, USA). Three burr holes were drilled through the parietal bone, and adenovirus was injected along three tracts with two deposits in each tract [5.0 and 4.0 mm ventral from the dura, with 2×10^6 expression-forming units (EFU) in 1 μl phosphate-buffered saline (PBS) per deposit]. The rate of injection was 0.5 μl/min, and the time interval of each deposit was 2 min. The coordinates of the injections were as follows: (1) 1.4 mm anterior and 2.6 mm lateral of bregma, (2) 0.4 mm anterior and 3.0 mm lateral of bregma, and (3) 0.8 mm posterior and 3.8 mm lateral of bregma. After injection, the needles were retracted at a rate of 0.5 mm/min.

Behavioral tests

Each rat was subjected to a series of behavioral tests by experimenters who were blind to the groups to which the rats had been assigned. The sensorimotor battery consisted of two tests that have each been used separately to evaluate various aspects of neurologic function.

Modified neurological severity score (mNSS). Neurological functions were assessed using the mNSS (Chen et al., 2001), which is composed of sensory (visual, tactile, and proprioceptive), balance (beam walking), motor (muscle status and abnormal movement), and reflex (pinna, corneal, and startle) tests. Each rat was graded on a scale of 0 to 18 (0, no impairment; 1–6, mild impairment; 7–12, moderate impairment; 13–18, severe impairment).

Accelerated rotarod. Deficits in motor function were measured using an accelerated rotarod (Bioseb, Chaville, France). The rotarod cylinder was accelerated from 4 to 40 rpm over 2 min in the pre-ischemic delivery or 5 min in the postischemic delivery. The test was composed of training and testing sessions. Training was performed for three consecutive days, and the rats that could stay on the cylinder for more than 110 s in the pre-ischemic delivery or 270 s in the postischemic delivery were used for our studies. During the test session, the rat was placed on the rotating cylinder, and the duration of time that the rat remained on the cylinder was recorded.

BrdU (5-bromo-2-deoxyuridine) labeling

BrdU (50 mg/kg in saline; Sigma-Aldrich, St. Louis, MO, USA) was injected intraperitoneally for seven consecutive days as indicated in Figs. 1, 4A. For immunostaining, the rats were anesthetized and then fixed by transcardiac perfusion with 4% formaldehyde/PBS (pH 7.4). The brains were immersed in 4% formaldehyde/PBS at 4 °C for 24 h, cryoprotected in 15–30% (w/v) sucrose/PBS, and cut into coronal slices of 40 μm thickness.

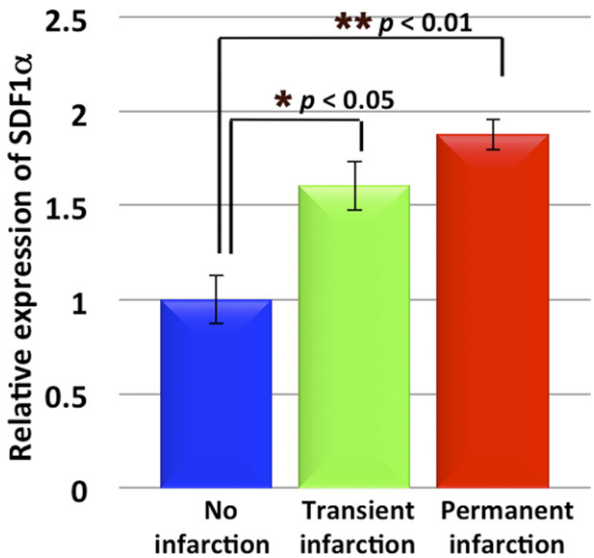

Fig. 1. Increased expression of endogenous SDF1α after stroke. Quantitative real-time PCR analysis was performed to compare the levels of endogenous SDF1α expression among the brains that were subjected to sham surgery or transient or permanent MCAO surgery. The value from the sham control was arbitrarily set to 1. A minimum of five independent PCR reactions were performed for each sample. $n=3$. Data are mean±SEM. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.