Improving Neurorepair in Stroke Brain Through Endogenous Neurogenesis-Enhancing Drugs

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Abstract
Stroke induces not only cell death but also neurorepair. De novo neurogenesis has been found in the subventricular zone of the adult mammalian brain days after stroke. Most of these newly generated cells die shortly after the insult. Recent studies have shown that pharmacological manipulation can improve the survival of endogenous neuroprogenitor cells and neural regeneration in stroke rats. As these drugs target the endogenous reparative processes that occur days after stroke, they may provide a prolonged window for stroke therapy. Here, we discuss endogenous neurogenesis-enhancing drugs and review the general status of stroke therapeutics in evaluating the field of pharmacotherapy for stroke.

Keywords
cerebral ischemia, drug treatment, neurogenesis, stem cells, subventricular zone, brain repair

Introduction
After the onset of ischemic brain injury, a series of time-dependent pathophysiological responses are activated. These reactions not only include excitotoxicity, apoptosis, leakage of the blood–brain barrier (BBB), and inflammation cell death but also endogenous neural repair (for details, please see the study by Brouns and De Deyn1). Some of these responses occur shortly after stroke and last for hours up to 1 to 2 d. One example is the apoptotic cascades at the ischemic site.2 Using a rodent distal middle cerebral artery occlusion (MCAo) model, it has been demonstrated that the density of terminal deoxynucleotidyl transferase 2’-deoxyuridine 5’-triphosphate (dUTP) nick end labeling (TUNEL) labeling in the ischemic cortex peaks on day 2 and returns to basal levels within 6 d after MCAo.3 Similarly, p53 messenger RNA (mRNA) or protein is upregulated shortly after stroke, which leads to p53-dependent programmed cell death in the penumbra.4,5 Because of the short duration of apoptosis/necrosis in the ischemic region, the efficacy of pharmacological treatment to reduce this mechanism is often restricted by its narrow therapeutic window. Stroke animals receiving p53 inhibitor treatment, given before or shortly after MCAo, had less brain infarction.6,7 P53 inhibitors given 3 h or later had no beneficial effect on neuronal cell loss7 and did not alter loco-motor behaviors.3 Taken together, these data suggest that the suppression of apoptosis in the ischemic-lesioned region must take place early after MCAo. This temporal restriction has limited the clinical potential for these drugs.

Cerebral ischemia also activates delayed endogenous repair processes. De novo neurogenesis was found in the subventricular zone (SVZ) of adult mammalian brain days after stroke.8 The kinetics of neural progenitor cells (NPCs) in the SVZ following ischemia has been examined using bromodeoxyuridine (BrdU) labeling.9 A robust increase in BrdU immunoreactivity in the SVZ occurred as early as 2 days after distal MCAo. The increase in BrdU immunoreactivity was sustained through 4 days after MCAo, started to decline between days 6 and 8, and returned to basal levels about day 10. These data suggest differential temporal windows of cell proliferation in the SVZ and cell death in the ischemic-lesioned zone. Targeting the survival of the

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endogenous NPCs in SVZ may enable a longer treatment window after the onset of stroke.\textsuperscript{3,9}

**Endogenous Neurogenesis-Enhancing Drugs**

Several candidate chemicals were recently developed to modulate survival, proliferation, migration, or differentiation of NPCs in the SVZ. Since these drugs aim at the endogenous reparative processes, which occur days after stroke, they may provide a prolonged window for stroke therapy. The following are examples of these drugs that may be potentially used for the delayed stroke therapy.

A. Pifithrin-\(\alpha\) (PFT-\(\alpha\)): Although stroke can activate the proliferation of NPCs in the SVZ, most of these cells die after injury.\textsuperscript{10} The NPCs in the SVZ express high levels of p53 protein.\textsuperscript{11} Increased TUNEL activity was found in the SVZ of stroke rats,\textsuperscript{7} indicating that apoptosis is also involved in the death of these NPCs. As the activation of NPC proliferation in SVZ occurs at a later stage,\textsuperscript{12} administration of PFT-\(\alpha\), a p53 inhibitor, starting from day 6 after MCAo, was found to enhance the survival of endogenous NPCs in the SVZ and improve motor function in stroke rats.\textsuperscript{3} These data suggest that inhibition of p53 may extend the survival of endogenous NPCs and alter biological outcomes days after stroke.

B. Trophic factors: Selective trophic factors have been shown to enhance neuroreparative activity after stroke. For example:

i. Bone morphogenetic protein 7 (BMP7). BMP7 is a trophic protein in the transforming growth factor-\(\beta\) (TGF-\(\beta\)) superfamily. BMP7 can promote DNA synthesis, as visualized by BrdU incorporation in mesencephalic-cultured neurons.\textsuperscript{13} Posttreatment with BMP7 enhanced the recovery of sensorimotor function in the impaired limbs,\textsuperscript{14,15} decreased body asymmetry, and increased locomotor activity from day 7 to day 14 after MCAo.\textsuperscript{16} BMP7 also increased BrdU immunoreactivity in the SVZ, lesioned cortex, and corpus callosum. The BrdU-positive cells were co-labeled with nestin and NeuN.\textsuperscript{17} These data support that BMP7 improves functional recovery through the proliferation of new neuronal precursors in the stroke brain.

ii. Brain-derived neurotrophic factor (BDNF). In nonstroke animals, BDNF has been shown to enhance migration of NPCs from the SVZ toward the olfactory bulbs.\textsuperscript{18-20} In stroke animals, systemic administration of BDNF enhanced the recruitment of NPCs into the lesioned site.\textsuperscript{21} Atorvastatin, a chemical that activates the expression of BDNF, enhanced migration of SVZ cells.\textsuperscript{9,22} Similarly, overexpression of BDNF through gene therapy via Adeno-associated virus (AAV) infection facilitated endogenous NPC migration from the SVZ and improved the functional recovery.\textsuperscript{23} These data suggest that administration of BDNF protein or increasing BDNF expression facilitates behavioral recovery through the enhancement of migration of NPCs from the SVZ in stroke animals.

iii. Other trophic factors: Several other trophic factors have also been reported to induce neurorepair in experimental stroke animals. For example, transplantation of neural stem cells or human umbilical cord blood CD34\textsuperscript{+} cells overexpressing glial cell line derived neurotrophic factor (GDNF)-enhanced neurogenesis.\textsuperscript{24,25} Infusion of GDNF increased cell proliferation in the SVZ\textsuperscript{26} and the recruitment of new neuroblasts into the striatum after MCAo.\textsuperscript{27} Other trophic factors, including hepatocyte growth factor, epidermal growth factor (EGF), and basic fibroblast growth factor (bFGF) have been shown to increase the number of BrdU-positive cells in the SVZ and improve endogenous neurogenesis in the stroke brain.\textsuperscript{26,28,30}

C. Cocaine- and amphetamine-regulated transcript (CART): CART is an endogenous peptide found in the brain. The expression of CART can be upregulated by oxygen–glucose deprivation in culture\textsuperscript{31} and focal cerebral ischemia or electroconvulsive shock in vivo.\textsuperscript{32} Treatment with CART before stroke reduces cerebral infarction in mice.\textsuperscript{31,33} Recent studies have shown that intranasal CART treatment from day 3 after MCAo enhanced neural repair by facilitating endogenous NPC proliferation and migration from the SVZ, enhancing reinnervation into lesioned cortex, and improving the functional recovery in stroke animals.\textsuperscript{7,34} In the SVZ, CART enhanced immunolabeling of BrdU, the NPC marker Musashi-1, and the proliferating cell nuclear antigen, as well as upregulated BDNF mRNA. In SVZ culture, CART increased the neurosphere formation. CART-mediated cell migration from SVZ explants was antagonized by anti-BDNF-blocking antibody. Using 1H-magnetic resonance spectroscopy, increases in N-acetylaspartate levels were found in the lesioned cortex after CART treatment in stroke brain. CART also increased the expression of growth associated protein 43 (GAP43) and FluoroRuby fluorescence in the lesioned cortex. These data suggest that intranasal CART treatment facilitates neuroregeneration in the stroke brain.\textsuperscript{9,34}

**Naturally Occurring Compounds as Agents for Enhancing Endogenous Neurogenesis in Stroke**

Dietary supplementation has been shown to stimulate endogenous brain repair mechanisms that afford neuroprotection
in stroke. Such dietary supplementation at a prestroke period exerts neuroprotection by reducing inflammation and elevating neurogenesis, thereby prophylactically preparing the brain in subsequent injury.35,36 The next step is to examine whether a poststroke dietary treatment is similarly effective in dampening the secondary cell death associated with stroke. Many of the components of the diet are naturally occurring compounds found in daily foods, such as polyphenols from blueberry and green tea and amino acids like carnosine, which are high in antioxidants and possess anti-inflammatory activity that decreases the damaging effects of reactive oxygen species in the blood, brain, and other tissues of the body.35,36 Accordingly, developing these dietary supplements as adjunct agents for the prevention or treatment of chronic diseases, including the secondary cell death in stroke, may be clinically relevant. We envision dietary supplementation as an adjunct therapy for stroke at acute, subacute, and even chronic periods.

An Overview of Recent Advances in Stroke Therapeutics

Until now tissue plasminogen activator (tPA) remains the gold standard treatment for ischemic stroke. Unfortunately, tPA has a very narrow therapeutic window of 4.5 h post-stroke, with its delivery beyond this time frame associated with serious adverse events, in particular hemorrhagic transformation (HT). Treatment strategies designed to abrogate the risk of HT and other complications associated with delayed tPA are likely to improve stroke outcomes. To this end, drugs that maintain the patency of the cerebrovasculature to avert delayed tPA-induced HT have attracted several lines of pharmacotherapy-based preclinical research. Noteworthy, among these drugs tested in preclinical stroke models that may confer protection of the cerebrovasculature include endogenous neurogenesis-enhancing drugs, including those discussed above.9–12,18–20,23,25,34 Other drugs examined as potentially exerting anti-HT features include those that preserve the BBB and those directly enhancing the brain vasculatures. However, despite encouraging laboratory evidence demonstrating amelioration of HT in stroke animals, optimization of the drug regimen, including the dose, timing, and route of administration, remains to be determined in order to translate these findings into clinical applications.

Conclusion

Current drug therapy for stroke is limited by narrow therapeutic windows. Several chemicals, peptides, or trophic factors have been reported to improve behavioral recovery when given after stroke.37–39 These drugs mainly target endogenous repair originated from the neuroprogenitor cells in the SVZ. This experimental approach may provide a new potential treatment strategy for stroke patients, enabling a longer treatment window after the onset of stroke, a disorder frequently logistically difficult to treat immediately after occurrence.

Authors’ Note

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