Neurogenesis, Angiogenesis, and MRI Indices of Functional Recovery From Stroke

Michael Chopp, PhD; Zheng Gang Zhang, MD, PhD; Quan Jiang, PhD

Abstract—This article analyzes the mechanisms underlying the potentiation of functional recovery poststroke by cell-based and pharmacologic agents, which amplify endogenous neurogenesis in the subventricular zone and angiogenesis in the border of the ischemic lesion in the animal. Discussion of the interaction between angiogenesis and neurogenesis is provided and data are described demonstrating a role for matrix metalloproteinases expressed in perinfarct vasculature as chemotactic for neuroblasts migrating from the subventricular zone. Monitoring angiogenesis and structural changes in the ischemic brain associated with functional recovery by means of MRI is described. We demonstrate that injured brain can be stimulated to promote angiogenesis and neurogenesis, which are coupled restorative processes that contribute to functional recovery from stroke and that MRI indices of these neurorestorative events are highly correlative with neurologic function and may be used in real-time monitoring of recovery from stroke. (Stroke. 2007;38[part 2]: 827-831.)

Key Words: functional recovery ■ MRI

Fewer than 5% of stroke patients receive recombinant tissue plasminogen activator. Thus, there is a compelling need to develop treatments of ischemic stroke designed specifically to reduce neurologic deficits, which can target the vast majority of patients.

The injured brain after onset of stroke in the experimental animal is driven to a quasidvelopmental state exemplified by the induction of an array of genes and proteins last seen in the embryonic and developing brain.1 This observation suggests that injured brain is primed for potentiation of neurorestorative processes. There are multiple ways to promote these restorative processes, including cell-based and pharmacologic therapies.2–10 The cell-based therapies that are under intense investigation include bone marrow mesenchymal cells, cord blood cells, fetal cells, and embryonic cells among others.4,11–14 In addition, there are many agents that trigger endogenous neurorestorative processes within the brain that may contribute to the reduction of neurologic deficits after stroke. Included among these agents are drugs that increase cGMP (phosphodiesterase 5 inhibitors, eg, sildenafil, tadalafil), statins, erythropoietin, granulocyte-colony stimulating factor, and minocycline.3,5–7,15,16 Experimental studies in laboratory animals suggest that cell and pharmacologic neurorestorative treatments may be of benefit when administered up to weeks after stroke. If these treatments were to be successfully translated to the stroke patient, the very narrow hour range window for treatment would be lifted, and therapy could be initiated well after the patient is stabilized, possibly months after ictus. Many of the noted treatments, both cell- and drug-based, have been used for other clinical indications and have superb safety records, which would foster translation to the clinical environment.

Importantly, these neurorestorative treatments amplify endogenous processes of brain plasticity, including neurogenesis and angiogenesis that may contribute to improvement in neurologic function after stroke.14,41 Neurogenesis after stroke is localized to tissue adjacent to the lateral ventricles and the dentate gyrus.17–26 Angiogenesis, the generation of new blood vessels, is most prominent in the ischemic boundary zone.27–31 Neurogenesis and angiogenesis are highly coupled and act to create restorative microenvironments within the ischemic tissue leading to improvement in neurologic function.32–34

Neurogenesis

In the experimental animal, stroke significantly increases the number of newly produced cells in the vicinity of the lateral ventricle13,14,22,24,35–39 and neurogenesis is present in the subventricular zone (SVZ) of patients with Huntington disease.40 A basic question is, what causes this amplification of neurogenesis in the SVZ? There are many molecular contenders for stimulating this process, including basic fibroblast growth factor, epidermal growth factor, insulin like growth factor 1, sonic hedgehog, and brain-derived neurotrophic factor.19,41–45 Increased production of new brain cells after stroke can also be attributed

Received August 17, 2006; accepted August 22, 2006.
From the Department of Neurology, Henry Ford Health Sciences Center (M.C., Z.G.Z., Q.J.), Detroit, Michigan; and the Department of Physics, Oakland University (M.C.), Rochester, Michigan.
Correspondence to Michael Chopp, PhD, Department of Neurology, Education & Research Building, Room 3056, Henry Ford Health Sciences Center, 2799 W. Grand Boulevard, Detroit, MI 48202. E-mail chopp@neuro.hfh.edu
© 2007 American Heart Association, Inc.

Stroke is available at http://www.strokeaha.org DOI: 10.1161/01.STR.0000250235.80253.e9

827
to a reduction in the G1 phase of the cell cycle.46 This reduction in cell cycle was associated with a reduction in expression of cyclic-dependent kinase inhibitors, including p27.47 The molecular primers for this increase in cyclic-dependent kinases are unknown. The observation of a stroke or cerebral injury-mediated increase in production of brain cells is also consistent with the concept that injured brain mimics an earlier stage of development.1

Cell Migration—Doublecortin and Matrix Metalloproteinases

Doublecortin is a marker for migrating neuroblasts.48 Cells generated in the region of the lateral ventricle migrate long distances to tissue adjacent to an infarct.13,22,24,35–39 What allows these cells to bore through the ischemic tissue to sites distant from their birthplace and how do these cells survive their long distance travel? One factor that can facilitate the migration of neuroblasts to remote regions of tissue is that these cells produce matrix metalloproteinases (MMPs), particularly MMP 2,49,50 MMPs degrade the extracellular matrix. Neuroblasts secreting MMPs can penetrate the continuum of the extracellular matrix and, like tumor cells, traverse a solid barrier to reside in the penumbral zone.

Migration of neuroblasts through the brain from the SVZ is unimpeded, and it appears that many cells survive the journey through regions of tissue alien from the birth source. We therefore asked the question whether these neuroblasts express proteins that are neuroprotective, proteins that facilitate the survival of these cells. Because doublecortin is a ubiquitous marker of migrating neuroblasts, we tested the hypothesis that doublecortin has a neuroprotective role in the migrating neuroblasts. SVZ cells derived from the rodent were subjected to oxygen glucose deprivation, mimicking some of the events after injury. These cells show significantly increased expression of doublecortin. Cells transfected with the doublecortin gene also exhibited robust and significant increase in survival when subjected to oxygen and glucose deprivation. Likewise, siRNA, which reduced expression of endogenous doublecortin in SVZ cells, made the cells significantly more vulnerable to stress induced by oxygen glucose deprivation. Thus, these data indicate that doublecortin is not only a marker for migrating neuroblasts, but also insulates neuroblasts from the potential dangers of migration.51

Neuroblast Migration and Vascular Signaling

In the normal noninjured rodent brain, neuroblasts are locked into a fixed migration pathway from the lateral ventricle to the olfactory bulb along the route of the rostral migratory stream.6,52,53 In sharp contrast, after stroke and injury, many cells deviate from this rigid path and migrate within the parenchyma to sites of injury.13,22,24,37–39 A fundamental question is, what are the molecular signals that dictate this rerouting to injured tissue? There is substantial literature demonstrating that chemokines, particularly stromal derived factor-1/CXCR4, contribute to the migration of these cells.54–57 CXCR4 is increased in neuroblasts, and stromal derived factor-1 is significantly increased in the cerebral tissue adjacent to the ischemic lesion.55–58 Do other factors target neuroblast migration? MMPs are strongly expressed, primarily in the microvasculature in the lesion boundary after stroke.49,50 We then asked the question, whether MMPs in the compromised vascular tissue adjacent to stroke also target neuroblasts to injured tissue? Using cell culture systems, we demonstrated that endothelial cells activated by a neurorestorative agent such as erythropoietin significantly increased the expression of MMP 2 and MMP 9 and significantly increased the migration of neuroblasts.49 MMP inhibitors greatly attenuated the endothelial cell mediated migration of neuroblasts. These data indicate that signals within the compromised microvasculature serve as a stimulus promoting neuroblast migration. In vivo data strongly support this hypothesis with the observations of expression of MMPs by the ischemic vasculature and the localization of migrating neuroblasts to this microvasculature.50,59 These data imply that the endothelial cells adjacent to injured tissue act as sentinels calling for help from the restorative properties of neuroblasts. The neuroblasts themselves produce angiogenic factors and MMPs,50,60,61 which close the loop of vascular sentinels calling for help from the restorative properties of neuroblasts.

MRI Monitoring of Plasticity and Recovery After Stroke

In the experimental animal, there are multiple interlaced neurorestorative processes activated after stroke and amplified with restorative treatment.3,5–7,62,65 These treatments revise brain architecture, including vascular changes associated with angiogenesis, neurogenesis, glial changes, reduction of scar tissue with treatment, increased presence of progenitor and mature oligodendrocytes after treatment, and changes in the white matter.14,11,27,65–67 Aspects of brain structural and angiogenic remodeling may be visible on MRI.12,67,68

MRI Indices of Angiogenesis

Angiogenesis may contribute to recovery after stroke.27,69–71 The question is whether angiogenesis is visible using MRI. Angiogenic vessels at early stages are permeable.27,72,73 We capitalize on this transient increase in vascular permeability and use it as a signal for new vessel formation.12 Vascular permeability can be quantified and detected using T1 indices of brain to blood transfer constants of extrinsic contrast agents such as gadolinium DTPA as well as intrinsic magnetization contrast methods and integrated multidimensional MRI techniques.12,67 In a recent study, we demonstrated that 1 to 2 weeks poststroke in the rodent treated with cell-based therapy, Ki, the transfer constant of Gd-DTPA was significantly increased. The signals were not attributed to edema as indicated by T1/T2 images. Histologic measurement of vascular density...
approximately 6 weeks later demonstrated a remarkable correspondence of vascular permeably and an increased vascular density corresponding to angiogenesis. These data demonstrate that newly formed vessels have MR signatures, and angiogenesis may be monitored using MRI.

**MRI Indices of White Matter Changes**

Treatment of experimental stroke with neurorestorative therapy evokes the production of white matter fibers and axons adjacent to the ischemic lesion. Diffusion tensor imaging and fractional anisotropy (FA) provide qualitative and quantitative indices, respectively, of white matter structure. Within tissue, isotropic diffusion of water reflects cavitation. Tissue with directionality in white matter tracks exhibits anisotropic diffusion characteristics, and these characteristics can be used as markers of structural change. In an animal model of stroke, we tested the ability of FA, which is a quantitative index of directionality, to identify cerebral tissue undergoing recovery after stroke and treatment with neural progenitor cells. The hypothesis was that neurorestorative treated recovery tissue exhibits higher anisotropy than recovery tissue in nontreated brain. Using T2 maps, the recovery area was defined by subtracting the area of the lesion at 5 weeks poststroke from the lesion area measured 1 day after stroke. Measurement of FA of these regions in both nontreated and treated stroke animals demonstrated a significant increase in FA in the recovery region of the cell-treated group compared with the nontreated group; likewise, there was a significant increase in the recovery region compared with the core of the lesion in both treated and control stroke animals. Multivariable analysis using FA and other MR parameters also showed a near unity correlation between neurologic recovery and MRI parameters. These data strongly indicate the power of MRI to monitor changes in brain associated with recovery of neurologic function. These methods are under active development in our laboratory. More sophisticated methods are being pursued, which allow dissection of white matter fibers with complex crossover geometry. Implementation of these methods will likely lead to improved analysis of brain tissue architecture related to neurologic functional recovery. This approach may open up opportunities for the management and the treatment of the stroke patient with potential application to neural injury and neurodegenerative disease.

There is a substantial body of literature supporting the role of angiogenesis and neurogenesis in mediating functional recovery after experimental stroke. Nearly all the neurorestorative agents that improve functional outcome after stroke increase angiogenesis and neurogenesis. Inhibition of neurogenesis in brain impairs neurologic function after stroke. These data imply a direct and causal relationship between neurogenesis, angiogenesis, and recovery of neurologic function after stroke. However, additional work is required to explicitly demonstrate that structural changes in brain, including neurogenesis and angiogenesis, potentiate recovery and are not simply secondary bystander events.

In summary, there is an opportunity to stimulate the brain’s own restorative capacity to improve neurologic function after stroke. There are both cell-based and pharmaceutical approaches that amplify these restorative reprocesses and lead to improved neurologic function in the experimental animal.


Neurogenesis, Angiogenesis, and MRI Indices of Functional Recovery From Stroke
Michael Chopp, Zheng Gang Zhang and Quan Jiang

Stroke. 2007;38:827-831
doi: 10.1161/01.STR.0000250235.80253.e9
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/38/2/827

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/