Brain Repair and Recovery from Stroke

a report by

Michael Chopp, PhD, Yi Li, MD, Jieli Chen, MD, Rui Lan Zhang, MD *and* **Zheng Gang Zhang, MD**

Neuroscience Institute, Henry Ford Hospital

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For decades, the overwhelming emphasis on the development of therapeutic interventions for the treatment of stroke has been in the area of neuroprotection, acute intervention to reduce the volume of cerebral infarction, and the sequellae of secondary cell death, whether by necrosis or apoptosis.[1,2] Concerted efforts to elucidate mechanisms of cell death were translated into the development of many neuroprotective agents, including antioxidants, n-methyl-D-aspartate (NMDA) antagonists, and anti-inflammatory agents.[1,2]

However, none of these agents has been proved clinically effective and the field of clinical trials in stroke neuroprotection is littered with failed and costly efforts.[1,2] The only 'effective' therapeutic approach was the development of thrombolytic therapy with recombinant tissue plasminogen activator (rtPa).[3] When administered within three hours after stroke, rtPa can improve outcome.[3,4] However, fewer than 5% of ischemic stroke patients in the US receive rtPa.[1–3,5] This is due to its short therapeutic window and potential adverse effects of hemorrhagic transformation.

For the sake of the stroke patient, we must shift the therapeutic paradigms. The focus of therapy should not necessarily be on the ischemic lesion destined to infarct, but on the remodeling of the intact brain and spinal cord to promote recovery of neurological function. In other words, treat the non-injured brain and not the infarct. The overemphasis on neuroprotection has been based on the erroneous assumption that the brain contains a fixed number of neurons and is difficult to remodel.[6,7]

However, since the 1960s it has been known that new neurons are generated in the animal brain.[8–10] Today, we know that the injured brain is highly malleable and the intact entire brain responds to injury and stroke by producing new brain cells (neurogenesis), new vasculature (angiogenesis and arteriogenesis), and new wiring (synaptogenesis and axonal growth), and these events collectively improve neurological function after stroke.[6,11,12] However, the majority of patients fail to regain full function after stroke, and more than 30% are left with severe disabilities.[7] To address this compelling clinical problem, it is necessary to amplify the endogenous neurorestorative response of the brain to stroke and injury in order to stimulate intrinsic neurorestorative pathways so that we can further improve neurological function after stroke.

Pre-clinical data demonstrate that after stroke the brain expresses an array of developmental genes and proteins—particularly in the boundary of the ischemic lesion—reminiscent of the developing brain.[13–15] We can capitalize on this attempted return to youth and amplify these restorative processes to rewire and restructure the central nervous system (CNS) in order to minimize loss of function.

In this article, we will focus on two complementary approaches of enhancing neuroplasticity and thereby promoting neurological function: cell-based and pharmacological therapies. Both restorative treatments improve functional outcome after stroke, with no reduction in infarct volume.

Cell-based Remodeling of Brain After Stroke—Concepts and Pre-clinical Studies

Cell-based therapy induces the recovery of function post-stroke by stimulating endogenous restorative mechanisms rather than by replacing infarcted tissue. When injected into the adult, the cells do not repopulate the adult brain tissue, regardless of whether they are bone marrow mesenchymal (MSC),[16,17] neurospheres,[18–20] umbilical cord blood,[21] or fetal and embryonic progenitor or stem cells.[22] Conversely, they produce an array of factors, including angiogenic and neurotrophic factors, that initiate the restorative cascade of recovery.[23] More importantly, these administered cells also act as catalysts to stimulate parenchymal cells—e.g. astrocytes, microglia, and endothelial cells—to produce the restorative factors that mediate brain remodeling and recovery of function.[24,25]

The vast majority of the many pre-clinical studies performed to date have employed cells injected directly into the brain[26,27] or administered via a vascular route that localizes to the region of cerebral injury.[16,28] Few of these injected cells express the parenchymal cell phenotype.[16] Functional improvement tends to be rapid and is often obvious within one week, which is clearly insufficient time for these alien cells to become neurons and successfully integrate into the brain circuitry.[16] At least for the adult, the

Michael Chopp, PhD, is Vice Chairman for Research in the Department of Neurology, Scientific Director of the Neuroscience Institute, and Zoltan J Kovacs Chair in Neuroscience Research at Henry Ford Hospital, Detroit. He is also a Distinguished Professor of Physics at Oakland University, Rochester. Dr Chopp is a Fellow of the American Heart Association (AHA), is on the Editorial Boards of *Stroke* and the *Journal of Cerebral Blood Flow and Metabolism*, and has chaired and been a member of National Institutes of Health (NIH) study sections. His research has focused primarily on the cellular and molecular biology of ischemic cell injury, the pathophysiology of stroke and traumatic brain injury, combination neuro- and vascular protective therapies for stroke, cell-based and pharmacological neuro-restorative therapies for stroke, traumatic brain injury, and neurodegenerative disease, treatment of glioma, and magnetic resonance imaging. Dr Chopp received his training in mathematical and solid state physics at New York University.

E: chopp@neuro.hfh.edu

use of cell-based therapy as a neurorestorative treatment is based on the ability of these cells to interact with parenchymal cells in such a way that the CNS is rewired and populated with new blood vessels and neurons, which are generated in response to the exogenously administered cells.

There are many options for cell-based therapies. Among the most tested in the adult are MSCs,[16] umbilical cord blood cells,[21] neuroblasts,[19,20] circulating progenitor endothelial cells,[29] adult stem cells,[30,31] and embryonic and fetal stem cells.[32,33] As a prototype of cell-based therapy, we will concentrate on MSCs. These cells can be administered by various routes including vascular,[16,28] intracerebral,[34,35] and intrathecal to induce remarkable recovery of function in a variety of stroke models, primarily in the rodent. Our preference has been to administer cells by an intravenous route. These cells target the injured or compromised microvasculature, localize to the ischemic border tissue, and encompass the lesion, where the cells stimulate recovery.[17,28] In direct contrast to neuroprotective strategies, cell- and pharmacologically based therapies can be administered days and weeks after stroke onset, with pre-clinical data demonstrating robust efficacy when MSCs are administered one month post-stroke.[36] We should also note that these treatments have been shown to be efficacious in models of hemorrhagic stroke;[37] thus, nearly all stroke patients can be treated. Functional benefit has also been demonstrated to persist in the rodent for at least one year post-treatment.[28] Male[16,17] and female[36,38] and young[16,17] and older[28,36] animals with stroke have robust functional improvement with cell-based therapies.

The mechanisms of action noted above likely encompass a tapestry of restorative events driven by the expression of trophic factors by the administered cells and responses by the endogenous parenchymal cells that remodel the brain, by vascular, neurogenic, neurite outgrowth, and synaptic alterations. Angiogenic events are highly coupled to neurogenesis and synaptic activity.[39–44] Newly formed vasculature expresses brain-derived neurotrophic factor (BDNF) and matrix metalloproteinases,[39,42] which act in concert to recruit neuroblasts from the subventricular zone to the site of vascular alteration. In turn, these neuroblasts induce angiogenesis and couple with the vessels to promote synaptic rewiring.[41,42] Angiopoietin 1 and its receptor Tie2 are also upregulated in the ischemic brain in response to MSC therapy, and contribute to the maturation and stabilization of this newly formed vasculature.[45]

Cells reduce scar tissue formation[28,36,46] and, importantly, reduce inhibitory glycoproteins. When inhibited, these proteins are permissive of neurite outgrowth and axonal remodeling in both the brain and the spinal cord.[47] There is evidence of axonal transcallosal rewiring in the contralateral hemisphere in response to cell-based therapy treatment.[38]

A somewhat neglected but obviously important area of interest is the response of the spinal cord to stroke and restorative cell therapy. Motor and somatosensory response requires communication with the spinal cord via the cortical spinal tract (CST).[48,49] Thus, the recovery of function may be associated with plasticity in the CST and the spinal cord. Anterograde and retrograde labeling of the CST demonstrates a remarkable pattern of neurite outgrowth from the intact to the denervated spinal cord, which significantly correlates with somatosensory functional recovery.[50] Retrograde labeling of bilateral forelimbs also demonstrates cross-connections in the contralateral and ipsilateral brain hemispheres amplified by MSCs. Downregulation of inhibitory glycoproteins may contribute to this robust rewiring in the brain and spinal cord.

Changes in white matter in response to either a cell or a pharmacological restorative therapy can be readily monitored using magnetic resonance imaging–diffusion tensor imaging techniques (MRI–DTI).[19,44] Tissue is cavitated, in which the diffusion tensor for water is isotropic. The more anisotropic the diffusion constant for water, the more structure is present in the tissue.[51–53] Water moves easily along white matter fibers, and these structural changes in white matter and axonal growth may become evident using direct thrombin inhibitors (DTIs).[51–53] Pre-clinical data demonstrate that cell therapy evokes white matter changes in the corpus callosum, the striatum, and the boundary region of the ischemic lesion, which are sensitive to the DTIs. Furthermore, significant correlations between functional recovery and a DTI-based parameter, fractional anisotropy (FA), may find clinical application.[19,44]

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Clinical Trials of Stroke with Cell-based Therapies

The first cell-based therapy for the treatment of stroke employed cells—the Ntera 2/ce.D1 human embryonic carcinomia-derived cell line—was injected into the brain of patients six months after stroke.[54] Twelve patients were treated: no cell-related adverse effects were reported and outcome measurements were consistent with a trend of improved neurological scores. Bang et al. employed autologous bone marrow mesenchymal cells from acute stroke patients.[55] Although this was a safety study, there was evidence of functional improvement. Additional trials for stroke are under way, and hopefully these early studies will spur application of this promising restorative therapy.

Pharmacological Restorative Therapies

Stimulating recovery of function is by no means the sole domain of cell-based therapies. Amphetamines have been tested as a treatment for stroke;[56] however, clinical trials have not shown evidence of benefit.[57] An angiogenic and neurotrophic agent—basic fibroblast growth factor (BFGF)—was tested in a phase II/III clinical trial of stroke patients.[58] The trial had to be terminated for safety reasons. However, there is a new generation of agents that can initiate the multiparallel cascades of neurorestoration and brain remodeling to reduce neurological deficits. Here, we discuss some studies with agents that are widely employed for other indications and have an excellent safety and efficacy profile for other diseases.

Phosphodiesterase 5 Inhibitors

Nitric oxide (NO) plays an important role in the developing CNS, with strong expression of enzymes for NO, such as neuronal and endothelial

NO synthase.[59,60] Treatment of stroke in animals with NO donors demonstrated robust therapeutic benefit, with improved functional recovery when the agent was administered one or more days after stroke.[61–63] NO increases cyclic guanosine monophosphate (cGMP), which is a major cellular second messenger.[59,60] Subsequently, we tested the hypothesis that the therapeutic benefit of NO donors may be attributed to increasing cGMP. One way to increase cGMP is to block its hydrolysis by phosphodiesterase 5 (PDE5).[64,65] Therefore, we employed PDE5 inhibitors (sildenafil and tadalafil) to treat experimental stroke in young and aged animals, and found a significantly improved functional outcome.[61–63,66] PDE5 inhibitors are widely used for the treatment of erectile dysfunction.[66] Functional benefit was evident in treatment from one to 30 days after stroke.[61–63,66] Brain plasticity was amplified with significant increases in angiogenesis, neurogenesis, and synaptogenesis.[61–63,66,67]

These potent pre-clinical data have led us to initiate a dose-tiered phase I clinical safety trial in stroke patients, with patients treated from three to seven days post-stroke. In a compassionate-use application, sildenafil has evoked remarkable recovery in a locked-in patient.[68]

Statins

Statins such as simvastatin and atorvastatin, among others, are in use worldwide for the treatment of elevated cholesterol. However, statins are pleiotropic and have benefits well beyond their reduction of low-density lipoproteins. They have been employed as neuroprotective agents for stroke,[69,70] and stimulate recovery of neurological function after experimental stroke, traumatic brain injury, and intracerebral hemorrhage.[71–76] Statins increase cGMP and NO, activate restorative signal transduction pathways such as PI3k/Akt, and stimulate the production of an array of angiogenic and restorative factors.[69–72,76] Animals with stroke treated with a statin one or more days post-stroke show substantial improvement in functional recovery with all of the concomitant indices of brain remodeling.[71] Based on these robust pre-clinical data, a phase II clinical trial for the treatment of stroke patients with statins[69] and a phase I trial for intracerebral hemorrhage are in progress.

Erythropoietin and Carbamylated Erythropoietin

Erythropoietin (EPO), a glycoprotein hormone produced in the kidney that regulates red blood cell production, and carbamylated EPO (CEPO) are neuroprotective in the acute treatment of experimental stroke.[77–81] EPO has been widely used to treat anemia and has found application as a supportive therapy in cancer patients.[82] In contrast to EPO, CEPO—a modified EPO—does not increase hematocrit.[83] A phase II clinical trial treating acute stroke patients with EPO has shown evidence of therapeutic benefit, and a phase III trial is under way.[84] These agents have also been tested for a restorative effect in the treatment of stroke. When administered 24 or more hours after stroke, EPO or CEPO enhances functional recovery and upregulates the indices of brain remodeling, which has been noted with other restorative therapies.[42,85–87] EPO increases cGMP, and both EPO and CEPO trigger signal transduction pathways evident in other restorative treatments (unpublished data). In addition to safety determination, additional pre-clinical work with CEPO likely has to be performed prior to entry into clinical trials. It is also important to test the effects of these agents pre-clinically in animals that received a thrombolytic agent, such as rtPA, so as to simulate in the laboratory all clinically relevant conditions.

Other Promising Restorative Agents

There are many more agents that are entering into the arena of restorative therapies. Neurotrophic factors and granulocyte colony-stimulating factors are rapidly being advanced as potential therapeutic restorative agents.[88,89] Recently, the benefits of high-density lipoproteins (HDLs) as restorative factors have been investigated, with evidence demonstrating that Niaspan®, a slow-release form of niacin that increases HDL, improves functional outcome when administered well after stroke onset.[90]

Conclusions

This review is not comprehensive. It simply indicates that the field of restorative neurology for the treatment of stroke is rapidly progressing, and that cell and pharmacological therapies can stimulate and amplify recovery of function in the injured brain. Hopefully, this paradigm shift to neurorestorative therapy treatment will find rapid and effective application in the treatment of stroke. ■

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