Review Article

Integrated Brain Restoration after Ischemic Stroke – Medical Management, Risk Factors, Nutrients, and other Interventions for Managing Inflammation and Enhancing Brain Plasticity

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Abstract

Brain injury from ischemic stroke can be devastating, but full brain restoration is feasible. Time until treatment is critical; rapid rate of injury progression, logistical and personnel constraints on neurological and cardiovascular assessment, limitations of recombinant tissue plasminogen activator (rtPA) for thrombolysis, anticoagulation and antiplatelet interventions, and neuroprotection all affect outcome. Promising acute neuroprotectant measures include albumin, magnesium, and hypothermia. Long-term hyperbaric oxygen therapy (HBOT) is safe and holds great promise. Eicosanoid and cytokine down-regulation by omega-3 nutrients docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) may help quench stroke inflammation. C-reactive protein (CRP), an inflammatory biomarker and stroke-recurrence predictor, responds favorably to krill oil (a phospholipid-DHA/EPA-astaxanthin complex). High homocysteine (Hcy) is a proven predictor of stroke recurrence and responds to folic acid and vitamin B_{12}. Vitamin E may lower recurrence for individuals experiencing high oxidative stress. Citicoline shows promise for acute neuroprotection. Glycerophosphocholine (GPC) is neuroprotective and supports neuroplasticity via nerve growth factor (NGF) receptors. Stem cells have shown promise for neuronal restoration in randomized trials. Endogenous brain stem cells can migrate to an ischemic injury zone; exogenous stem cells once transplanted can migrate ("home") to the stroke lesion and provide trophic support for cortical neuroplasticity. The hematopoietic growth factors erythropoietin (EPO) and granulocyte-colony stimulating factor (G-CSF) have shown promise in preliminary trials, with manageable adverse effects. Physical and mental exercises, including constraint-induced movement therapy (CIMT) and interactive learning aids, further support brain restoration following ischemic stroke. Brain plasticity underpins the function-driven brain restoration that can occur following stroke.

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Introduction

Stroke, the third leading cause of death in the United States and the leading cause of adult disability, can be catastrophic for the afflicted individual. Because current therapies for stroke have extremely limited effectiveness, most stroke patients never fully regain lost function. Although total restoration of function following stroke remains a rare accomplishment, advances in neurobiology are setting the foundation for a new era of brain restoration. This review summarizes the management of acute stroke, identifies risk factors for secondary prevention of stroke recurrence, and explores the research advances toward effective brain restoration.

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Stroke: A "Brain Attack"

Stroke is sometimes called "brain attack" to more aptly describe its insidious but rapid onset. Signs of stroke include:[1,2]

- sudden, unexplained dizziness, trouble with walking, loss of balance or unsteadiness
- confusion, trouble speaking or understanding communication
- unexplained weakness or numbness of the face, arms, or legs, or on one side of the body
- loss of vision in one or both eyes
- sudden, unexplained severe headache

Stroke is a disruption of blood supply to the brain, whether due to blood vessel occlusion (ischemic stroke; IS) or rupture with bleeding (hemorrhagic stroke). As the blood supply becomes compromised, lack of oxygen and nutrients limit cell function and survival. A zone of cell death is created, bordered by a zone of damaged cells, the so-called penumbra. Within the total lesion zone, oxidative, inflammatory, and probably also excitotoxic cascades become activated and threaten to spin out of control.

Strokes that do not totally destroy vital brain zones can nonetheless cause ongoing impairment of motor, sensory, or processing pathways and consequent degradation of quality of life. Seizures, symptomatic hemorrhaging, and brain swelling occur in up to one-third of patients soon after stroke onset.[3] Acute management of stroke is not straightforward and requires skilled personnel. The patient who does not receive prompt and specialized care can succumb to the spread of brain damage.

In 2007, the American Heart Association and its division, the American Stroke Association, co-published an authoritative, comprehensive set of updated guidelines for the acute management of stroke (herein abbreviated "Guideline"). The 51-page Guideline, prepared by a panel of experts appointed by the American Heart Association Stroke Council's Scientific Statement Oversight Committee, was also endorsed by the American Academy of Neurology and appeared in its most complete form in the journal *Circulation*.[3] The Guideline's primary goal is to provide an overview of the current evidence on evaluation and treatment of adults with acute ischemic stroke, with recommendations covering management from the first contact with emergency services personnel through initial admission to the hospital.

Approximately 80 percent of strokes are ischemic in origin,[4] since they result either from thrombus *in situ* or an embolism of distant origin. Ischemic stroke is the primary focus of this review, with occasional comment on hemorrhagic stroke. Ischemia is an insufficiency of oxygen and nutrient supply due to circulatory impairment. Most ischemic strokes have hemorrhagic involvement.[3] In the United States, 700,000 people suffer a full-blown IS each year, 200,000 in individuals who have suffered a previous stroke.[5] Estimates of the number who experience transient ischemic attacks (TIAs) are much higher. Year by year, the pool of post-stroke candidates for brain restoration continues to expand.

Strokes differ from TIAs only in degree. According to the American Heart Association/American Stroke Association (AHA/ASA), when neurological symptoms continue beyond 24 hours the event is defined as a stroke, when less than 24 hours it is a TIA.[5] With modern brain imaging some TIAs found to have a lesion are then classified as strokes. TIAs are important stroke predictors, with 90-day stroke risk as high as 10.5 percent and the greatest stroke risk apparent in the first week following the TIA.[6]

Among 30-day survivors of a first stroke, about half survive five years.[7] After the immediate imperatives of the acute phase, reducing the chance of another stroke must take priority. Epidemiological studies and prospective clinical trials have identified the predominant risk factors for recurrent stroke. An in-depth AHA/ASA document is available with guidelines for prevention of a second stroke.[5] Implementation of these recommendations, both by stroke survivors with support networks and by the community as a whole, is a prerequisite for successful stroke rehabilitation.

Current Medical Management of Stroke

Brain restoration ideally begins once the stroke has been detected, relying on efficient medical management of the acute stage. According to the Guideline,[3] however, acute stroke care is limited in its ability to contain brain damage. Thrombolysis (thrombus breakdown), the only intervention currently approved by the

U.S. Food and Drug Administration (FDA), does not work adequately for many patients and is effective only when intervention occurs within three hours of symptom onset. Few healthcare centers are sufficiently prepared to offer this intervention with the rapidity and degree of interdisciplinary coordination the Guideline requires. In addition to recanalization (reopening) of the occluded vessel(s) by thrombolysis, other conventional interventions include management of other circulatory complications by surgery or the use of anticoagulant drugs.

Acute Care: Thrombolysis

The Guideline states that thrombolysis by the intravenous (IV) administration of recombinant tissue plasminogen activator (rtPA) is the most beneficial proven intervention for emergency stroke treatment.[3] Approved by the FDA in 1996, it is still the only approved treatment for acute IS. But due to the strict time constraints for intervention, inadequate readiness at some hospitals, substantial risk for intracerebral hemorrhage, restrictive patient selection criteria, and other factors such as transport to the treatment facility, only one- to three-percent of stroke patients receive rtPA treatment.

Intravenous administration of rtPA, specified in the Guideline at precisely 0.9 mg/kg IV, maximum 90 mg,[8] is associated with improved outcomes for patients who can be treated within three hours of stroke onset. Earlier treatment (i.e., within 90 minutes) gives a statistically more favorable outcome (odds ratio of 2.11; 95% confidence interval, 1.33-3.55) for favorable outcome at three months compared to placebo. In comparison, the odds ratio for intervention at 90-180 minutes is 1.69 (95% confidence interval, 1.09-2.62). The major risk from rtPA intervention is hemorrhage. In the definitive trial with 624 patients published in 1996 by the National Institute for Neurological Disorders and Stroke,[8] symptomatic hemorrhage occurred in 6.4 percent of subjects given rtPA versus 0.6 percent of placebo subjects. Current overall expectation for hemorrhage is about six percent.[9]

Despite its preferred status at the FDA and within the cardiovascular healthcare community, rtPA has significant limitations. When it works, intravenous or intra-arterial rtPA takes at least 15-30 minutes to reopen an occluded major vessel such as the middle cerebral artery (MCA), and no evidence exists that other thrombolytic agents have faster action.[3] Doppler studies suggest only a 30-percent complete recanalization rate for MCA occlusion after rtPA, a 48-percent partial recanalization rate, and a 27-percent reocclusion rate.[10] Combining rtPA with transcranial ultrasound significantly improved the rate of complete recanalization, accompanied by marked clinical recovery. Another option under investigation is lower-dose IV rtPA followed by lower-dose intra-arterial rtPA delivered through an ultrasonic catheter.[3]

The Guideline mandates screening criteria for patients who could reasonably be treated with rtPA, as well as details of the specified administration technique (refer to Tables 11 and 12 in the Guideline).[3] It emphasizes that while other thrombolytic drugs are currently under investigation, none has been established as effective or as a replacement for rtPA. It does suggest that patients with ischemic stroke secondary to occlusion of the MCA, who are evaluated within six hours of symptom onset and ruled ineligible to receive IV rtPA (because of recent surgery, for example), may be candidates for thrombolysis by the intra-arterial route using urokinase as the primary alternative to rtPA.[11]

According to AHA/ASA experts, the foremost challenge in the management of acute ischemic stroke remains to "recanalize" the affected zone, that is, to re-establish blood supply in order to preserve tissue viability. In the Guideline, surgical procedures are rejected as not proven effective and bearing substantial risk for harm.[3] The Guideline does suggest potential benefit from endovascular interventions – emergency angioplasty and stenting, mechanical disruption of the clot, or extraction of the thrombus. It critiques the trials of angioplasty (with or without stenting) in combination with thrombolytic agents to achieve recanalization. Mechanical clot disruption using an endovascular photoacoustic device can speed recanalization. Recently developed for clot extraction is a device christened MERCI from the Mechanical Embolus Removal in Cerebral Ischemia Trial;[12] although approved by the FDA, the clinical utility of this device remains unclear.

Anticoagulant and Antiplatelet Drugs

While acknowledging that physicians have used anticoagulants to treat acute ischemic stroke for more than 50 years, the Guideline questions their routine use. The Guideline rules out intravenous heparin within the first 24 hours as not effective and carrying unacceptable risk of hemorrhagic side effects. In fact, aspirin is ranked over heparin as an antiplatelet drug.

The Guideline accepts that interventions that down-regulate platelet aggregation can reduce the risk for recurrent stroke in patients with acute IS, but recommends aspirin as the only antiplatelet drug adequately validated for this purpose. The Guideline recommends oral aspirin (at a 325 mg/day initial dose) within 24-48 hours after stroke onset for treatment of most patients.[3] Aspirin is classified as adjunctive to rtPA, not a substitute for it, and should not be started earlier than 24 hours after stroke onset. Clopidogrel, while promising, requires further research.

Vasodilators

Beyond 24 hours of the stroke onset, the Guideline states that vasodilators such as pentoxifylline and pentofylline given intravenously for 3-7 days are unlikely to improve outcome. This is not to say that increasing blood perfusion does not have benefit. The AHA/ASA experts discuss "preliminary and small clinical studies" in which increased blood pressure to the damaged penumbra zone seemed to improve outcome. The rationale for these studies is that the penumbra zone has impaired autoregulation of blood pressure and that induced hypertension increases perfusion into the zone (as witnessed by brain imaging) to potentially salvage endangered tissue.[13] The Guideline does not recommend the use of drugs to induce hypertension for this purpose except in clinical trials or in exceptional cases involving close neurological and cardiac monitoring.[3]

Neuroprotection: Time is Brain

Characteristic of stroke is the rapid progression of damage in the affected zone(s) of brain tissue; the first hours are crucial. Research in animal models suggests most of the tissue damaged by cerebral infarction is beyond salvage within 1-2 hours.[14] A variety of agents working via diverse mechanisms have proven neuroprotective in such models but so far none has worked in human trials (see the Guideline[3] for a review). Investigators suggest this is because neuroprotection must be initiated within two hours of stroke onset, as the animal model experiments typically are designed.[14] A popular adage in stroke research is "time is brain," meaning every moment that passes in acute stroke means more brain function lost.

While the ongoing search for patentable neuroprotectants produced hundreds of candidates that failed to prove clinically significant in randomized controlled trials (RCTs), three interventions unlikely to be patentable have shown capacity for neuroprotection. These are IV albumin, IV magnesium, and hypothermia.

Albumin: Safe and Effective Neuroprotectant

Supplementation with human serum albumin is a safe and effective established treatment for patients whose circulating albumin is low.[3] Albumin also is known to reduce brain swelling in patients with hemorrhagic stroke or other brain trauma.[15,16] In several animal models of IS, albumin infusion reduced infarct size and swelling, improved local perfusion to zones of critical blood flow reduction, and improved neurological and behavioral functions.[17] In an RCT conducted by Shin et al,[15] patients with moderate-to-severe IS in the area of the middle cerebral artery were randomized within 12 hours after symptom onset. The albumin group was divided into three subgroups: (1) "albumin low-dose, early" received 0.63 g/kg body weight over two hours; (2) "albumin high-dose, early" received 1.26 g/kg over four hours (both were completed within the first 12 hours of symptom onset); or (3) "albumin high-dose, late" received 1.26 g/kg over four hours, 12-24 hours after symptom onset; the control group received saline.

In this trial patients were subjected to thorough evaluations including magnetic resonance imaging (MRI), vascular studies, and blood tests.[15] Neurological changes were evaluated using a modified National Institutes of Health Stroke Scale (mNIHSS),[18] initially at admission and subsequently on days 1, 3, 7, and 14. Functional recovery was evaluated using the Barthel Index (BI) and modified Rankin Score (mRS) at admission and on days 7, 14, 30, and 90. Infarct volume was measured by MRI diffusion-weighted imaging at admission and at 72-96 hours after onset.

Of the 49 patients who completed the Shin trial,[15] 18 were controls, eight were in the albumin low-dose early, 13 were in the albumin high-dose early, and 10 were in the albumin high-dose late subgroups. Although infarct volume continued to increase in controls and albumin groups, when measured at 72-96 hours after onset the volume increase was significantly less in the albumin-treated patient group as a whole (26.0% increase versus 67.2% increase for the controls, p=0.012). Among the albumin subgroups, only the high-dose patients treated early showed significantly lower infarct increase (which notably was only 1-2% over the volume at admission), whereas the controls averaged almost a 100-percent increase. The neurological scores for the albumin patients evaluated via mNIHSS were significantly more improved than the controls at days 3, 7, and 14. The functional analyses using BI and mRS showed significantly higher functional independence in the albumin group at day 90, with the high-dose albumin given early working best.

Albumin's neuroprotective effects can be explained via several mechanisms. This protein has a prolonged circulating half-life (about 20 days), and its high molecular weight keeps it in the blood vessel lumen, drawing in water and thereby expanding blood volume.[3,15,17] Albumin is also a potent antioxidant, a large molecule carrying a high density of cysteine groups.[19]

Albumin clearly has strong benefit in acute IS, without troublesome adverse effects. Besides having the advantage that it can be applied anytime within 24 hours of stroke onset, as shown in the Shin trial,[16] albumin is cost-effective compared to other in-hospital interventions.

Intravenous Magnesium Sulfate

Ionized magnesium has demonstrated effective neuroprotection in animal models of cerebral ischemia, excitotoxic injury, head trauma, and spinal cord damage.[20] Magnesium sulfate is a ready source of ionized magnesium with an established safety and efficacy profile in myocardial ischemia.[3] An international RCT – the Intravenous Magnesium Efficacy in Stroke (IMAGES) trial – suggests magnesium has neuroprotective potential for acute ischemic stroke.[21]

The IMAGES trial produced 90-day survival and disability data on 2,386 patients treated with IV magnesium sulfate started within 12 hours after stroke onset (16 millimoles over 15 minutes, then 65 millimoles over 24 hours). Although it found no statistically significant overall benefit, it did show a trend toward protection for patients with noncortical stroke.[21] Given the pressing need for stroke interventions, this was sufficient to justify the Field Administration of Stroke Therapy-Magnesium (FAST-MAG) trial.[20] An open-label trial prelude to FAST-MAG found dramatic early recovery in 42 percent of patients infused within two hours of stroke onset.[22] At 90 days post-stroke onset, good global function was achieved by 69 percent of all patients and by 75 percent of patients infused with magnesium sulfate within two hours of stroke onset.

FAST-MAG is a large, controlled trial with the statistical power to detect modest treatment effects of IV magnesium sulfate for very early stroke. FAST-MAG is being conducted primarily in the field – magnesium sulfate is easily administered and can be given over a wide dosage range without harm.[20] This unique trial hopes to demonstrate "pre-hospital initiation" whether magnesium infusion therapy, mostly via paramedics, can be effective in halting or slowing the ischemic cascade in most patients within those first crucial two hours. If this bold field trial proves to improve stroke outcome, it could usher in a new era of acute stroke management since rtPA cannot realistically be administered in the field.

Hypothermia: Already a Good Track Record

Hypothermia slows cerebral metabolism and protects neurons subjected to acute ischemia. This is a potent intervention that improves neurological outcomes after cardiac arrest and has been used to treat patients with severe brain edema.[23] Because the optimal body temperature for stroke management is not yet clear, caution must be taken since excessive cooling could precipitate adverse effects such as hypotension, cardiac arrhythmias, or infections.[3] Nonetheless, pilot studies suggest hypothermia might prove feasible for acute stroke.[24,25] Furthermore, after applying hypothermia and IV magnesium in animal experiments, Meloni et al suggest concomitant use of both interventions in patients should improve the overall neuroprotective outcome.[26]

Other Possible Neuroprotectants

Other candidates for neuroprotection mentioned in the AHA/ASA Guideline include mannitol, a safe agent for which the data is still inconclusive; cerebrolysin, which one small study found is safe and might improve outcomes;[27] and erythropoietin, which is discussed in a later section on hematopoietic growth factors.

The Acute Phase and Beyond: Hyperbaric Oxygen Therapy

Originally developed as a treatment for the "bends" in divers and later applied to carbon monoxide-poisoned patients and to wound healing, hyperbaric oxygen therapy (HBOT) involves bathing the individual in 100-percent oxygen at greater than normal atmospheric pressure (for references to the early literature, see Neubauer 1980[28]). This causes additional oxygen to dissolve in the blood, thereby increasing the amount of oxygen available to the cells – particularly brain cells in the case of stroke. HBOT can be safely instigated in acute stroke patients within hours after stroke onset. Despite controlled trials and hundreds of published case series and individual reports, HBOT remains outside the mainstream of medical stroke management.

The quality of the research on HBOT is spotty. In 2005 Carson et al evaluated four RCTs and another trial of HBOT for stroke.[29] While they concluded the best evidence showed no benefit, they also concluded that because of the stage of patients enrolled (acute, subacute, or chronic), the documentation of type and severity of stroke, and considerably varying dosages of HBOT, the negative results could not be generally assumed and further good quality studies were needed. Veteran HBOT investigators agree.[28,30]

In 2003 Rogatsky et al analyzed dosing patterns in clinical reports on HBOT for acute IS dating back to 1969 (total, 265 patients).[31] A total dose coefficient was developed and compared against the trial's efficacy, defined as improvement in percentage of patients up to 100 percent. The dose coefficient was calculated as the product of partial oxygen pressure (ATA, "atmospheres absolute units of the intrabarochamber pressure of oxygen") x time of exposure (hours) x number of exposures. A dose coefficient of 1.0 would be a Unit Medical Dose (UMD). For 14 patients classified as acute in the 1995 Nighoghossian study (a study that failed to find efficacy),[32] they calculated (1.5 ATA x 0.65 hour x 10.0 average exposures) and arrived at a UMD value of 9.7. After calculating UMD values for 10 other studies the researchers found a close correlation (0.92) between level of efficacy and UMD value and determined a threshold of 30-32 UMD for 100-percent efficacy – the minimum UMD of HBOT at which all patients should derive benefit.

The Rogatsky dose-efficacy correlation helps explain why some HBOT regimens failed to show efficacy in acute ischemic stroke.[31] In addition to the Nighoghossian study,[32] seven of 10 other studies below 30 UMD failed to reach 100-percent efficacy. The researchers also suggested that a total dose of 2-3 UMD per day should not result in undue toxicity.

Rockswold et al published an extensive 2007 literature review of HBOT for traumatic brain injury (TBI).[30] Citing 95 references, extensive evidence of HBOT efficacy, its mechanism(s) of action, and safety are reviewed, and the design of planned RCTs on severe TBI patients is described. Although not directly pertinent to stroke, this review does provide additional rationale for applying HBOT to stroke rehabilitation. The most likely mechanisms of HBOT's apparent benefits for TBI logically apply to ischemic stroke – improved mitochondrial function and improved oxygen utilization leading to enhanced cerebral metabolism.

Rockswold asserts that placing severely brain-injured patients in a hyperbaric oxygen chamber at 1.5 ATA for 60 minutes is a very low-risk procedure, proven to be free of potential brain or lung damage. This corresponds to a UMD of 1.5; therefore, to attain 32.0 total UMD at this exposure rate, 21 exposures are required, approximately the minimum number of exposures suggested by most experienced operators. The experience of one high-volume clinic with hundreds of stroke and other brain-injured patients suggests hundreds of exposures per year at 1.25-1.75 UMD are safe and efficacious.[33] According to the International Hyperbaric Medicine Association, in the United States HBOT is delivered 10,000 times each day at 800 locations.[34]

As suggested by its apparent success for TBI,[30] HBOT has considerable potential for stroke rehabilitation. In 1990 Neubauer et al used HBOT to reactivate what they termed "idling neurons" in the penumbral

zone of a right parietal lobe infarction in a 60-year-old woman who had a stroke 14 years earlier.[35] They used Single Photon Emission Computed Tomography (SPECT) imaging to document this and other HBOT recoveries.[33] Whether significant improvement is possible for every stroke patient remains to be convincingly demonstrated. However, advances in neurobiology since 1990 indicate the brain's plasticity or capacity to adapt has been underestimated, even in advanced age and with severe injury.[36]

Preventing Stroke Recurrence by Managing Risk Factors

For the stroke patient who survives the acute phase, secondary stroke prevention (stroke reoccurrence) is essential in order to restore brain function. Identifying and managing the risk factors for stroke can contribute to this goal.

As detailed by the AHA/ASA Guideline,[5,37] risk factors for secondary stroke prevention overlap those for primary prevention. In the case of certain risk factors – hypertension, diabetes, elevated cholesterol and triglycerides, smoking, heavy drinking, obesity, lack of exercise – effective management is relatively well established[5] and bears no detailed examination here. Other factors are more technically challenging but well recognized – symptomatic intracranial atherosclerosis, atrial fibrillation, and heart disease, for example.[5] Other risk factors for stroke recurrence are newly validated and require discussion. One that is highly predictive of stroke recurrence is C-reactive protein (CRP), an inflammatory biomarker.

C-Reactive Protein and Stroke Risk

Much, if not all, of the brain damage caused by stroke is a consequence of inflammation. CRP, an "acute-phase protein" is produced mainly in the liver, circulates in the blood, and is reflective of inflammation. As a thoroughly validated biomarker for cardiovascular health, it serves as a sensitive predictor of stroke risk.[38]

The advent of a highly sensitive blood test for CRP (hs-CRP)[38] was the technical breakthrough necessary to make CRP measurement routine. The hs-CRP test has sufficient precision to allow for correlation with cardiovascular and cerebrovascular disease risk. The power of the hs-CRP biomarker to predict adverse cardiovascular events (myocardial infarction, revascularization intervention, sudden death, or stroke) is now judged stronger than any of the cholesterol measures.[38,39]

CRP is produced in response to stimulation by the "pro-inflammatory" cytokine interleukin-6 (IL-6).[40] CRP levels accurately predict the risk of first stroke as well as risk for stroke recurrence.[40,41] In a 1999 study, hs-CRP level >10.1 mg/L within 72 hours of stroke predicted increased mortality over the ensuing four years.[42] Another study determined that hs-CRP level >15 mg/L at discharge was significantly associated with occurrence of a new "vascular event" or death at one year (95% confidence interval, 2.8-20.0). For those in the highest tertile of CRP the risk of an event within one year is 55 percent.[43] In a 2003 study, those in the highest quintile of CRP, measured at least three months after the first ischemic stroke or TIA, had significantly increased risk of subsequent stroke.[44] The strong power of blood CRP levels to predict stroke is consistent with stroke having a major inflammatory component.[40,45]

Monitoring conventional risk factors such as cholesterol remains valuable, although half of all heart attacks and strokes in the United States occur in people who do not have abnormal cholesterol or triglyceride levels.[46] An in-hospital study of 231 consecutive middle-aged IS cases found CRP measured at admission strongly predicted survival over eight days' hospitalization.[45] The threshold for increased risk was hs-CRP above 18 mg/L; for each additional 1 mg/L elevation the risk of death increased 20 percent (95% confidence interval, 1.09-1.30; $p<0.001$). Thus CRP's predictive power offers a valuable option for tracking mortality from acute stroke, as well as risk of recurrent stroke (or other adverse cerebrovascular or cardiovascular events).

The Importance of Inflammation in Stroke

Inflammation is an endogenous protective mechanism by which immune cells wall off the damaged brain tissue from surrounding uninjured zones, destroy dead or mortally injured cells, and repair the extracellular matrix.[47] All the brain's cell types, including microglia (macrophages resident in brain tissue), astrocytes, neurons, oligodendrocytes, and endothelial cells, participate in inflammatory responses.

Inflammation is normally a beneficial, self-limiting process that is active just long enough to stabilize the damaged zone and initiate a subsequent healing process. Although a stroke event invariably initiates inflammation, where the ischemic insult is severe the inflammatory response can be so substantial that it proves difficult to terminate.[48] Inflammatory cascades of oxidants, eicosanoids, cytokines, and chemokines are initiated with the onset of the ischemic insult. These effect the local inflammatory response and induce immune cells to migrate from outside the brain. Normally this integrated immune-inflammatory process brings the damage under control and triggers inflammation resolution.

Resolution of inflammation is a poorly understood yet crucial issue for stroke outcome. Brain tissue is not particularly well equipped with antioxidant defenses, so reactive oxygen species and other free radicals/oxidants that exude from inflammatory cells threaten tissue viability in the vicinity of the damage zone. For this immunologically aggressive phase to end additional mediators must be produced to calm the activated cells. The events that trigger this switch are poorly understood, but certain key mediators have been identified, the best studied of which are the omega-3 metabolites.[48]

Long-chain Omega-3s Help Control Brain Inflammation

The molecules that mediate inflammation are derived largely from long-chain fatty acids (LCFAs), especially arachidonic acid (AA) (C20:4, omega-6), eicosapentaenoic acid (EPA) (C20:5, omega-3), and docosahexaenoic acid (DHA) (C22:6, omega-3).[48] The functional localization of these LCFAs is within the cell membrane system.[49] They are covalently bonded to phospholipid molecules, which constitute the bulk of the cell membrane's lipid bilayer. From this position LCFAs can be catabolized to produce smaller messenger molecules that either amplify immune or down-regulate cell activity.

The omega-6 AA and the omega-3s DHA and EPA have differing influences on inflammation. AA tends to yield metabolites that amplify the inflammatory response, while DHA and EPA favor resolution of inflammation. Higher levels of DHA and EPA in membranes also down-regulate the protein cytokine messengers, probably through down-regulation at the gene level. Dietary intake raises omega-3 levels within brain cell membranes and creates a biochemical tilt away from AA metabolites. Dietary DHA also may up-regulate antioxidant defenses.[50]

New research on DHA has generated findings that clarify how it protects against inflammation. DHA is metabolized to substances known as resolvins, protectins, and neuroprotectins. As their names imply, these are the most potent LCFA metabolites for promoting inflammation resolution and protecting brain tissue from further damage through other degradative mechanisms.[48,49,51] Considering the typical Western diet greatly favors AA over DHA and EPA intakes, it would seem prudent to ensure the stroke patient has adequate intake of these omega-3 fatty acids, once the possibility of stroke-related hemorrhage in the brain has been ruled out.

Krill "Oil" Modulates Inflammation and C-Reactive Protein

Krill is a shrimp-like crustacean that thrives in the frigid ocean waters off Antarctica. Krill can manage this hostile environment partly because their cell membranes have a high concentration of long-chain omega-3 fatty acids.[49] The multiple carbon-carbon double bonds in these omega-3s (six in DHA, five in EPA) contribute electron mobility that makes krill cell membranes very fluid and therefore better able to resist very cold temperatures. DHA and EPA are anchored in the krill membranes exclusively via phospholipids, aptly termed omega-3 phospholipids (for details, see Kidd, 2007[49]).

Antarctic krill (*Euphausia superba*) can be processed to yield an "oil" that is mostly a complex of omega-3 (DHA and EPA) phospholipids and the carotenoid astaxanthin, responsible for its red color. Some omega-3 triglycerides are also present. Each of these krill oil constituents has anti-inflammatory actions. In randomized, controlled trials, krill oil demonstrated improved lipid profiles,[52] down-regulated inflammation symptoms,[53] and lowered CRP.[54] The vital lipids found in krill oil can be considered a "next-generation" omega-3 dietary supplement, one with benefits that encompass but overarch those of fish oil. By down-regulating pro-inflammatory gene activity, dietary DHA and EPA also help down-regulate interleukin-1 (IL-1), a cytokine that can promote brain inflammation.

Interleukin-1 and Brain Inflammation

Cytokines are small protein messenger molecules, the most studied for stroke being IL-1, an important mediator of the acute phase of brain inflammation. It is very low in the healthy brain, but rapidly becomes up-regulated when the brain is subjected to ischemia, hypoxia, trauma, or other inflammatory stimuli.[55] In rodents, within minutes after the brain is made ischemic, IL-1 release begins, first from the microglia then from the other cell types. As IL-1 rises, neuron death markedly accelerates.[56] Conversely, deletion of the genes that code for IL-1 reduces brain damage by 80 percent.[57]

For acute ischemic stroke patients, abnormal IL-1 elevation has negative clinical impact. Patients who have increased blood levels of messenger RNA for IL-1 tend to have a worse neurological outcome.[55] High circulating IL-1 in turn elevates circulating IL-6, chemokines, and other acute phase inflammatory mediators also associated with poor stroke prognosis.[58]

Several approaches are under investigation for managing IL-1 in stroke. IL-1 acts via membrane receptors (IL-1R), which can be blocked by a receptor antagonist (IL-1RA). In a randomized trial for acute stroke IL-1RA readily crossed the blood-brain barrier, was safe to use, and seemed to afford some benefit, particularly for patients with cortical infarcts.[59]

Homocysteine, Independent Risk Factor for Stroke Recurrence

Homocysteine (Hcy) is a sulfur-containing, thiol amino acid with a sulfhydryl group, generated mainly from the metabolism of methionine and homeostatically regulated by enzymes that rely mainly on vitamins B_6, B_{12}, and folic acid as essential cofactors. Multiple epidemiological studies in the United States, Europe, and Japan link high circulating Hcy to increased risk for stroke.[60-62] However, prospective clinical trials that used folic acid and vitamins B_6 and B_{12} to lower Hcy did not find lowered stroke risk. This has led to controversy as to whether Hcy actually can cause stroke,[63,64] with some investigators contending the epidemiological links may be artifactual.

The first linkage of Hcy with stroke came from observations that individuals with genetic inabilities to metabolize Hcy had very high urine levels (homocystinuria) and manifested circulatory damage early in life; about a dozen such inborn defects are known.[63,65] In 1962 McCully suggested even moderately elevated plasma Hcy might accelerate atherosclerosis.[66] Subsequent reports in the *New England Journal of Medicine* documented that one-third of patients with premature atherosclerosis showed elevated urine Hcy,[67] and 42 percent of patients with premature vascular disease showed elevated plasma Hcy.[68]

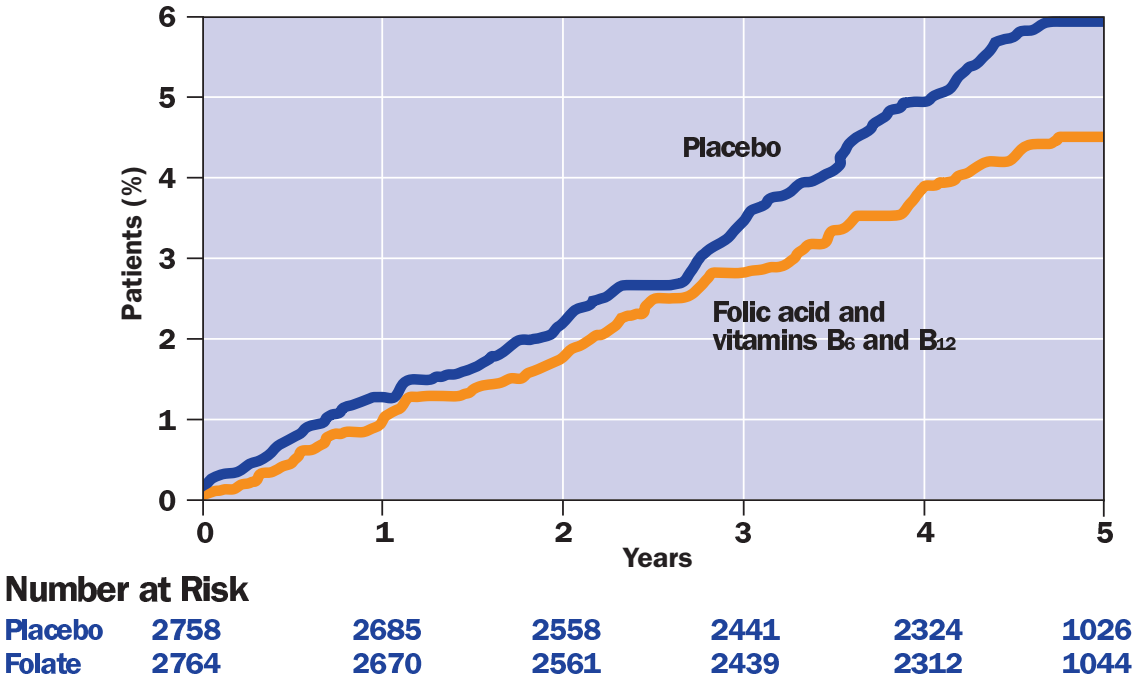
Advancing age, vitamin deficiencies, renal failure, and a number of pharmaceutical drugs can raise plasma Hcy (for references see Spence[63]). There are many plausible mechanisms by which elevated circulating Hcy could cause vascular damage. Elevated plasma total homocysteine (tHcy) is associated with abnormal elevations in oxidative stress, blood coagulability, and cholesterol synthesis; abnormally reduced HDL-cholesterol; and impaired endothelial function.[63,66-69] These and perhaps other obscure mechanisms help explain why elevated plasma tHcy may increase the risk for stroke.

Scientific consensus as to terminology is as follows. Plasma tHcy is a complex mix of free homocysteines (fHcy) and bound homocysteines (bHcy). The fHcy includes the thiol-reduced homocysteine, the free homocysteine, and various free mixed disulfides of homocysteine with cysteine and other thiols. The bHcy is almost all bound to proteins, especially albumins.[70]

B Vitamins, Homocysteine, and Stroke Risk

In prospective RCTs, B vitamin supplementation (folic acid + vitamin B_{12} + vitamin B_6) that reduced plasma tHcy levels did not reduce stroke incidence.[63,64] But case-control and other population studies of patients with vascular disease show tHcy is a strong, graded, and independent predictor of cardiovascular events, including stroke.[71-73] Plasma tHcy above 10.2 micromol/L was associated with two-fold greater risk of stroke,[71] and above 20 micromol/L with a nine-fold increase in risk.[72] The Hordaland Homocysteine Study, which tracked 18,000 men and women for five years, found that individuals with elevated tHcy at baseline were at heightened risk of morbidity, cognitive decline, and death during that subsequent five-year period.[73] Spence critically reviewed these data in an attempt to reconcile this apparent paradox.[63]

Figure 1. Effect of Vitamin Therapy to Lower Homocysteine on Risk of Stroke in the HOPE-2 Trial



Number at Risk

Placebo	2758	2685	2558	2441	2324	1026
Folate	2764	2670	2561	2439	2312	1044

From: Spence J. Homocysteine-lowering therapy: a role in stroke prevention? *Lancet Neurology* 2007;6:830-838. Reprinted with permission from Elsevier. Copyright 2007.

In the Heart Outcomes Prevention Evaluation 2 (HOPE-2) study,[74] the combination of folic acid (2.5 mg/day), vitamin B_6 (50 mg/day), and vitamin B_{12} (1 mg/day) versus a placebo for an average of five years failed to reduce cardiovascular death. However, the trial did show a significant reduction in stroke risk (relative risk 0.75; 95% confidence interval, 0.59-0.97; p=0.03). The investigators attribute this finding to chance because they could not find a biological explanation for how stroke could benefit from B vitamin therapy when other vascular diseases seemingly did not. Spence disagrees, contending this finding is likely real because the brain is more susceptible to thrombotic accident than the heart.[63]

Spence[63] argues that, whereas most myocardial infarctions are due to plaque rupture in a coronary artery, usually creating an *in situ* thrombosis, cerebral infarcts (ischemic strokes) occur most often from distant embolisms transported to the site.[74] To support this case he also notes high tHcy quadruples the risk of stroke from atrial fibrillation,[75] one source of distant embolism.

To emphasize his point, Spence reproduces a figure from the HOPE-2 trial showing the relative risk of stroke between the vitamin-treated and the placebo group diverging steadily with time (Figure 1). Spence makes a persuasive case that if these two lines were statistically inseparable or otherwise due to chance, they should criss-cross or remain close together rather than unerringly diverge.

Vitamin B_{12}: A Possible Key to the Homocysteine Paradox

In the Vitamin Intervention for Stroke Prevention (VISP) trial,[76] 3,680 patients who had experienced a non-disabling ischemic stroke were randomly

assigned to two groups. Both groups received the same multiple-vitamin preparation, which supplied the FDA recommended daily intakes of the other vitamins but was lacking in folic acid, vitamin B_6, and vitamin B_{12}. The low-dose group received in addition, 20 mcg/day folic acid, 200 mcg/day vitamin B_6 (as pyridoxine), and 6 mcg/day vitamin B_{12}. The high-dose group received 2.5 mg/day folic acid, 25 mg/day vitamin B_6, and 400 mcg/day vitamin B_{12}. At the end of the two-year trial neither group showed significant reduction in stroke recurrence. Spence asserts the negative trial outcome could be due to the B_{12} "high" dose being too low. According to Spence, one of the VISP trial investigators, many patients, including some in the high-dose group, had very low baseline serum B_{12} levels. All were periodically tested, and those found to have low serum B_{12} were given monthly B_{12} injections. Spence suggests this defeated the purpose of the study – to have a low-dose B vitamin control group. His group recalculated the VISP data, excluding patients who received vitamin B_{12} injections and some others with kidney problems known to be less likely to respond to vitamin therapy. The data from 2,155 participants showed the high-dose vitamin therapy significantly reduced stroke, coronary events, and death compared with the low-dose group. This re-analysis demonstrates that those patients who could absorb vitamin B_{12} (from a baseline B_{12} level at or above 322 picomol/L) and who received the "high-dose" combination, experienced one-third fewer strokes than the low-dose vitamin group.

Folic Acid Monotherapy Reduces Stroke Risk

Based on the methyl cycle rationale that folate is the major metabolic recycler of homocysteine to methionine,[16] a number of RCTs and other less well-controlled trials have tested folic acid as a monotherapy for stroke prevention. The most recently published meta-analysis concludes folate is effective for stroke. Analysis of eight RCTs demonstrated a statistically significant 18-percent reduction in stroke risk (relative risk 0.82; 95% confidence interval, 0.68-1.0; p=0.045).[77] Trials that went longer than three years showed a markedly greater risk reduction of 29 percent (p=0.001). A correlation between the degree of homocysteine reduction and the amount of stroke risk reduction was also seen; trials with greater Hcy reduction showed greater stroke risk reduction.

Despite the fact that folate alone reduces stroke risk, a combination of B vitamins is probably the better approach for three reasons. First, there are multiple roles for folate in one-carbon metabolism, which suggest its supply could become limiting to metabolism;[16] second, vitamin B_{12} is an essential cofactor for the remethylation of Hcy to methionine by folate;[16] and third, Spence's critique of the VISP trial suggests a likely role for vitamin B_{12} in lowering stroke risk.[63] Hence, using folic acid in combination with vitamin B_{12} may be superior to folic acid alone for stroke prevention.

Nutrients for Recovery After the Acute Phase

Only two nutrients have been clearly established for stroke recovery after the acute phase – citicoline and glycerophosphocholine.

Citicoline

Citicoline (also called cytidine diphosphate choline, cytidine diphosphocholine, CDP-choline) is a nucleotide and an energy-activated form of the essential nutrient choline. Citicoline consists of choline linked to cytidine by a diphosphate bridge. Citicoline is well absorbed when taken by mouth,[78] and following absorption into the intestinal endothelium it is hydrolyzed to choline and cytidine, which enter the circulation and cross the blood-brain barrier.[79] Citicoline as a dietary supplement has been investigated for hemorrhagic stroke in a small pilot study, with nonsignificant outcome;[80] and for acute IS in four RCTs.[81-84]

A 2002 meta-analysis[85] analyzed the data from the four RCTs on oral citicoline for acute IS.[81-84] All were performed in the United States and used various doses of oral citicoline (500, 1,000, or 2,000 mg per day) or placebo. In all cases, citicoline was begun within 24 hours after stroke onset, dosing ended at six weeks, and efficacy analysis was performed at three months. To be included in the meta-analysis, patients had to be 18 or older and have had a moderate-to-severe stroke (mild stroke was judged likely to spontaneously recover and mask any response to citicoline). The final patient total was 1,372; 789 on citicoline and 583 on placebo. Citicoline was associated with a significantly greater global recovery at three months (odds ratio, 1.33; 95% confidence interval, 1.10-1.62; p=0.0034).

Global recovery was defined as any degree of recovery on three separate assessment scales – the mNIHSS, the functional BI, and the global mRS. Global recovery was achieved by 25.2 percent of patients on citicoline compared with 20.2 percent of patients on placebo. The highest favorable response was obtained at the highest dose of 2,000 mg/day.

In this meta-analysis, citicoline when compared to placebo at three months significantly improved the probability to recover activities of daily living by 29 percent and the probability to recover functional capacity by 42 percent, but the increase in neurological recovery was not significant.[85] Citicoline had no effect on three-month survival; its significant adverse effects included increased anxiety and leg edema. The investigators concluded, "Although the capacity of citicoline to rescue ischemic tissue may be limited, its safety profile likely provides a favorable risk-to-benefit ratio.... A new trial to confirm these results should be conducted." A large, multicenter RCT of citicoline for acute ischemic stroke is now underway.[86]

Glycerophosphocholine

Like citicoline, glycerophosphocholine (GPC, alpha-GPC, choline alfoscerate, choline alphoscerate) is also a well absorbed and highly bioavailable source of choline. GPC has been administered to almost 3,000 stroke patients in five stroke trials.[87-91]

Each GPC stroke trial used the same protocol. Patients were started within 10 days after stroke onset. The trials consisted of two phases: during the first phase of 28 days, generally in hospital, GPC was given intramuscularly at 1,000 mg/day. During the second phase, from day 29 to day 180, GPC was given orally at 1,200 mg/day (400 mg three times daily). The single largest multicenter trial was conducted at 176 centers in Italy and included 2,044 patients.[88] During phase 1, the patient's medical history was taken, physical and neurological examinations conducted, and level of consciousness graded via the Mathew Scale (0-100, with 0 being clinical death and 100 being perfect consciousness).[92] Those too unconscious to be evaluated were excluded (Mathew Scale score <35). In phase 2, the Mini-Mental State Examination (MMSE) was used to evaluate cognitive function, and the Crichton Geriatric Rating Scale (CGRS) and the Global Deterioration Scale (GDS) were used to evaluate overall deterioration (see Barbagallo Sangiorgi et al[88] for references).

At the end of the six-month trial, using the patients as their own controls, the investigators found GPC significantly helped more than 95 percent of patients, and without serious adverse effects. In phase 1 the Mathew Scale score improved an average of 15.9 points ($p<0.001$). The MMSE was significantly improved at day 90 ($p<0.001$), then further at day 180 ($p<0.001$). Both the CGRS and GDS deterioration scores were significantly improved at day 180 ($p<0.001$ for both scales). Overall, GPC was judged by 78 percent of investigators as "very good" or "good," by 17 percent as "moderate," and by five percent as having "poor" or "no" efficacy.

Parnetti[93] performed a meta-analysis of the three stroke trials completed up to 2001[87,88,91] as part of a broader assessment of GPC for neurological diseases. She concludes all three trials produced similar results; GPC improved ischemic stroke recovery by a "clinically relevant" 20-30 percent in the first phase, and the second phase produced additional, clinically relevant improvements of 12-21 percent assessed by MMSE, CGRS, and GDS scales.

Cardiac bypass and other open-heart surgery results in as much as a 50-percent risk for memory loss and other cognitive brain damage. Some patients recover within days or weeks, others suffer permanent disability. In a double-blind trial, 20 recovering open-heart patients were randomized to receive GPC intravenously at 1,000 mg/day for one month, then intramuscularly at 1,000 mg/day for another five months or placebo.[94] Neuropsychological testing using the Wechsler Memory Scale determined GPC slowed memory deterioration significantly over placebo after one month ($p<0.05$). GPC also improved memory over the subsequent five months while the placebo group experienced ongoing memory decline ($p<0.05$).

GPC has potent neuroprotective properties. The most relevant to stroke are findings from a rat brain ischemia model, in which GPC given after arterial occlusion surgery protected the ischemic zone almost completely from damage.[95] GPC's degree of protection surpassed cerebrolysin, piracetam, and vinpocetine in this IS model. GPC is a unique osmotic protectant, especially against urea buildup[96] and demonstrates trophic activity via conservation of brain nerve growth

factor receptor density.[97] GPC is used in Europe to treat head trauma and other acute brain injuries in addition to stroke.[93]

Brain Regenerative Capacity: A Change in Perspective

Until recently, researchers in the field of stroke rehabilitation have been pessimistic, limited in their perspective by the prevailing scientific dogma. This dogma maintained that the human brain has very limited capacity to recover from structural and functional damage; therefore, stroke rehabilitation could merely hope to salvage damaged circuits that were still structurally intact. The patient was expected to adjust to living without the functions lost to stroke. As basic and clinical research spanning the last three decades began overturning this dogma, stroke rehabilitation entered a new, far more optimistic era.

Neurobiology researchers found the human brain cortex has anatomically identifiable sensory and motor ("sensorimotor") zones that manage the limb and other body functions. At first these nerve circuits were thought to be very specific for each zone, but now it is clear these brain zone "maps" are actually quite changeable. Elegant experiments were done in which a zone was knocked out by a mechanical or chemical lesion or inputs to a zone were changed from the sensory input source, and it was found when one zone is removed from action often an adjoining zone can substitute,[98] resulting in broader usage of the concept of "neuroplasticity."[99]

Brain Plasticity Regenerates Lost Function

The scientific dogma that held the central nervous system was not capable of regeneration was attributed to a very limited capacity for generating new cells,[100,101] the presence in the tissue of active inhibitors of regeneration,[102] and the scarring seen to ensue following damage.[102,103] In 1998 conclusive evidence emerged that the adult human brain makes new nerve cells.[100] These new cells can be induced to become functional. This breakthrough, combined with the steadily accumulating evidence of adaptability of established sensorimotor circuits, means the human brain can functionally regenerate enough to improve quality of life.

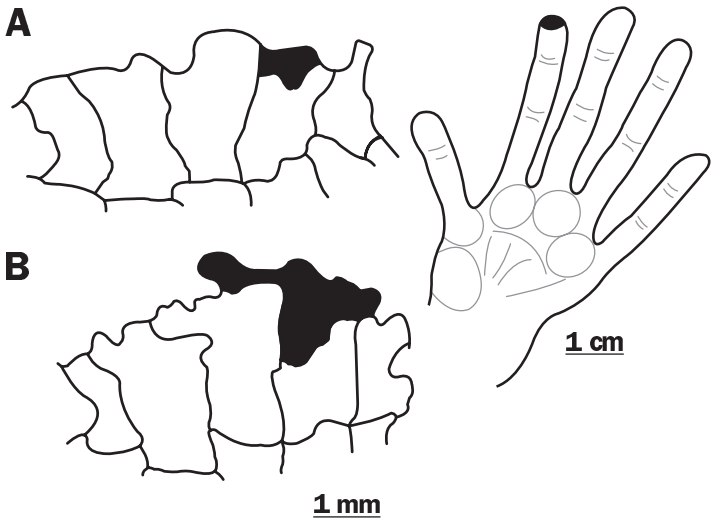
Brain plasticity refers to the brain's lifelong capacity for physical and functional change. This capacity explains how experience induces learning throughout life.[36,99] Over the last quarter century, pioneering plasticity researcher Michael Merzenich spent much of his early career in the laboratory placing tiny electrodes in brain neurons. What he found opened a new era in neurobiology; i.e., the brain's cortex is adaptable and changeable in response to manipulation of stimuli from the outside.[98,99]

By performing painstaking cortical mapping experiments, Merzenich et al documented the human brain cortex has a highly dynamic structural and functional topography. Neuron networks in functional zones continually adjust functional commitments when adapting to incoming information.[98,104] A classic example is when two adjacent fingers of a monkey are taped together over weeks, the fingers become one finger functionally; i.e., one functional unit for cortical "somatosensory area 3b."[104] The model appears to be that neurons receiving more stimulation make more connections, while neurons receiving less or no stimulation lose connections, sometimes becoming structurally atrophied. Cortical nerve network adaptability is likely the mechanism for the neuroplasticity that underlies stroke rehabilitation.[99]

Researchers mapped the somatosensory cortex of two monkey species, training the monkeys in finger retrieval of small objects (Figure 2).[105,106] Xerri identified the brain area associated with input to the fingers as cortical somatosensory area 3b. By creating microlesions in area 3b, essentially mini-strokes localized to the most functional zones of area 3b, the monkeys lost retrieval skills. The monkeys were retrained and over several weeks to months reacquired the fine somatosensory skills. When area 3b was remapped the researchers found new zones of area 3b had become involved. A new link had emerged in adjoining area 3a and further away, area 1 had doubled its zone of responsibility for fingers. Xerri concluded this was an example of cortical "substitution" and "adaptive plasticity" in recovery from stroke.

The monkeys did not show adaptive plasticity in the opposite ("contralateral") brain hemisphere, perhaps because the lesions were tiny and highly localized. Sophisticated Positron Emission Tomography (PET) and electroencephalogram (EEG) monitoring show recovering stroke patients display substitution and adaptive

Figure 2. Monkey Somatosensory Cortex/Finger Input[106]



The zone of monkey somatosensory cortex receiving input from a finger expands over time as that finger is intensively trained in tactile stimulation; A: before training, B: after training.

From: Jenkins W, Merzenich M, Ochs M, et al. Functional reorganization of primary somatosensory cortex in adult owl monkeys after behaviorally controlled tactile stimulation. *J Neurophysiol* 1990;68:82-104. Reprinted with permission.

plasticity in the cortical hemisphere affected by stroke, but also can display recruitment of additional circuits from the contralesional hemisphere.[107] This recruitment pattern in recovering stroke patients resembles that seen in healthy subjects facing challenges of complexity. The investigators concluded that to reacquire function lost from stroke is a challenging task.

The Potential of Stem Cells in Brain Plasticity

The early pioneers of brain mapping, using passive tools such as light microscopes to examine histologically processed tissue, did not find the mitotic figures that indicate cells in the division process. In 1998 Eriksson et al, using far more advanced imaging and staining technology, found newly produced neurons in the hippocampus of adult humans.[100] This finding established that the brain has stem cells that can differentiate into nerve cells by a process termed neurogenesis. From the evidence available, neurogenesis in humans probably does not occur in the brain cortex and may be restricted to the dentate gyrus of the hippocampus.[108] Neural stem cells (NSCs), however, can be harvested from elsewhere in the human brain, placed in cell culture, and induced to divide. This technique is termed expansion and relates to the potential for commercial scaling-up of production as outlined in Table 1.

It is estimated that a single NSC can form enough cells for 40 million brains.[109] NSCs generate both neuronal and glial cells in culture and, when transplanted into rodent brains, are incorporated into circuits and adopt both cell forms. They can be grafted, injected, or otherwise delivered to the body and are able to "home" to sites of injury. Human NSCs have been transplanted into rat and primate brain with genes for glial-derived neurotrophic factor (GDNF), a growth factor important for neurogenesis. Once transplanted they secreted GDNF for three months, and the experimental brain damage was partially ameliorated.[109]

Mesenchymal stem cells (MSCs) originate in the bone marrow.[109] On hard substrates they differentiate into bone cells, but on soft substrates they tend to differentiate into neurons.[109] MSCs are readily grafted into brains of other species (allogeneic transplantation).[110] In rat IS, human MSCs have a dose-response effect whether applied intravenously, intra-arterially, or intracerebrally.[111] Intravenous transplantation improved functional outcome when given one month after the ischemic insult,[112] qualifying as true neuroprotection. In another experiment, human MSCs were transfected with a gene to secrete brain-derived neurotrophic factor, then given intravenously to rats 3-6 hours after ischemia. The gene-doped MSCs showed better efficacy than MSCs not doped.[113] Autopsy of humans after bone marrow transplantation discovered bone marrow MSCs had homed to the brain and become neurons.[109]

A phase I trial examined autologous MSCs for IS.[114] Thirty patients were randomly assigned, five

Table 1. Proposed Cell Therapies for Stroke[109]

Cell type	Safety	Ethical Concerns	Scalability
Mesenchyme stem cells (MSC) (autologous)	Excellent (Phase I trial)	None	Poor
MSC (allogeneic)	Very good; host rejection possible	None	Excellent
Umbilical cord stem cells	Very good	None	Very good
Adult progenitor cells	Very good	None	Excellent
Neural stem cells	Good but largely unknown	Minor (depends on source)	Moderate

Adapted from: Hess D, Borlongan C. Stem cells and neurological diseases. *Cell Prolif* 2008;41 (Suppl 1):94-114.

to receive MSCs and 25 as controls. Autologous MSCs were delivered intravenously at a dose of 100 million within one month of ischemic stroke. The treatment proved safe, and a trend toward functional improvement was noted.

A subset of MSCs, the multipotent adult progenitor cell (APC) is also effective in a rodent stroke therapy model.[109] Intracerebral transplantation one week after cortical stroke improved sensorimotor function. These cells are effective in such small numbers they are thought to be trophic, i.e., growth supportive for host cells, rather than becoming neurons in the host brain. The same may be true for all known stem cell types.

Human umbilical cord stem cells (HUCS) delivered intravenously are also reported to be effective in animal models of cerebral ischemia.[109] It is unlikely many of these cells reach the host brain, yet they promote formation of new blood vessels (angiogenesis) and new circuit formation (neurogenesis) in brain tissue. Limited physician experiences with human umbilical cord therapies outside the United States reportedly have been positive.[115]

Although stem cells are now eligible for clinical trials, safety remains a concern. While earlier small trials with fetal stem cells caused adverse effects,[116] extensive animal studies with NSCs, MSCs, APCs, and HUCS have found no safety problem. Table 1 lists currently proposed cell therapies for stroke (the total list for neurological diseases is much longer).[109] Cell therapies for stroke are particularly attractive because they are practical to deliver and can improve stroke outcome even when applied days after the stroke event, in contrast to the three-hour therapeutic window for rtPA. Cell therapies have trophic effects, stimulating endogenous repair in the damaged brain. MSCs and HUCS are especially scalable and easily commercialized. Future clinical trials will establish whether stem cells can restore human brain function after stroke.

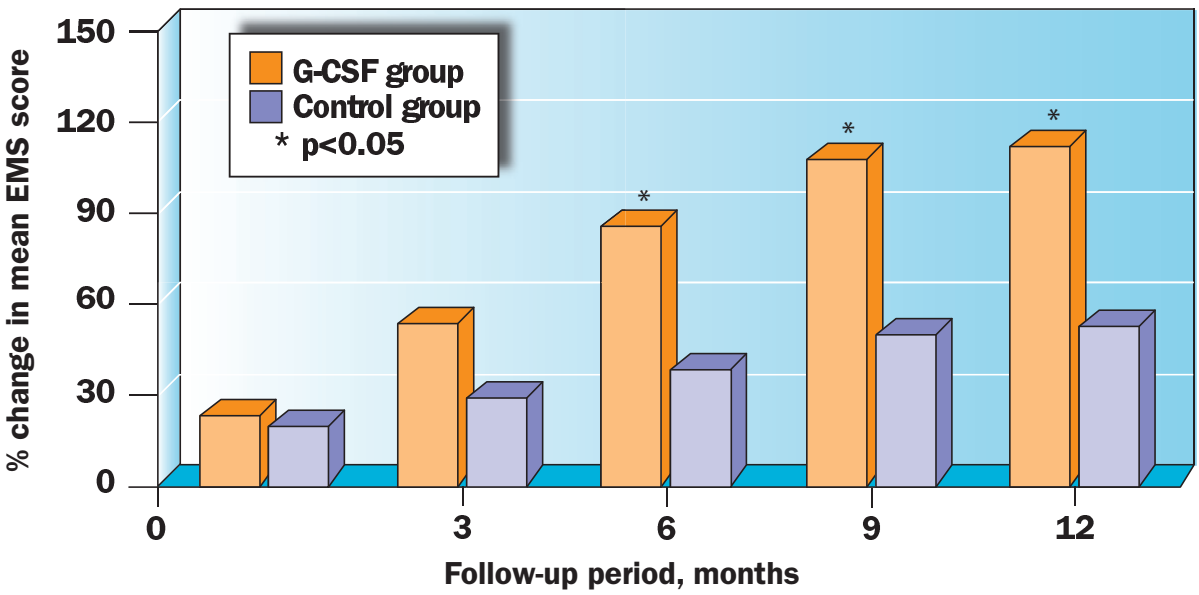
In animal stroke models, IS triggers stem cells to migrate to the site of injury.[116] Such stroke-induced neurogenesis has also been observed on autopsy of the brain of an 84-year-old patient days after a stroke.[117] A large number of NSCs and new blood vessel-forming cells were observed only around the region of infarction. An increased number of NSCs also were observed in the normally neurogenic region of the lateral ventricular wall.

Reminiscent of this human case is a landmark rat experiment that involved experimental ischemic injury to the striatum.[118] This resulted in amplification of stem cell production in the subventricular zone and subsequent migration of these cells to become mature neurons in the striatum. Interestingly, these stem cells would normally have become olfactory bulb neurons. As with the above patient, a neurogenic response to injury in the rats occurred that targeted the stroke-damaged zone.

Growth Factors Support Brain Circuit Restoration

The brain carries a number of growth factors, collectively termed neurotrophins (NTs). The list of known NTs includes nerve growth factor, the first growth factor to be discovered; brain-derived neurotrophic factor; glial-derived neurotrophic factor; ciliary neurotrophic factor; and neurotrophins, generically labeled NT-3, NT-4/5, and NT-6. Growth factors are proteins that control synaptic function and plasticity and sustain neuronal cell survival, morphology, and differentiation.[119,120] Overall, NTs tend to be

Figure 3. Neurological Improvement from G-CSF in Stroke Patients



Adapted from: Shyu WC, Lin SZ, Lee CC, et al. Granulocyte colony-stimulating factor for acute ischemic stroke: a randomized controlled trial. *CMAJ* 2006;174:927-933.

up-regulated by brain injury (including ischemia) and exercise and down-regulated by stress.[101] Although essential to neurogenesis and overall brain plasticity, none has entered prospective clinical trial for stroke. The two growth factors tested in stroke clinical trials are hematopoietic growth factor erythropoietin and granulocyte colony-stimulating factor.

Erythropoietin

Erythropoietin (EPO) is an FDA-approved hematopoietic growth factor that has receptors in the brain[121] and has been in widespread clinical use as an anti-anemia drug. After many promising animal studies, a human pilot study – the Goettingen EPO-Stroke Study – examined EPO for stroke.[122] Patients with MCA stroke were recruited. The protocol first involved a safety study (13 patients), followed by a double-blind trial (40 patients). EPO given intravenously readily crossed the blood-brain barrier, was proven safe, and demonstrated significantly better outcomes compared to placebo. EPO patients experienced significantly less neurological deficit, better restitution of brain function, and significantly smaller lesion size than controls.

A subsequent EPO trial for hemorrhagic stroke produced inconclusive results because it was compromised by too small a sample size.[123] EPO administration began 20 hours after stroke onset; whereas, in the Goettingen study, EPO began five hours after onset.[122] Twenty hours may have been too long to stem the damage. A larger, multicenter trial of EPO is in progress.[124] EPO can have hypertensive effects that increase risk of stroke, and its inappropriate use has been linked to the deaths of 18 cyclists from cerebral or myocardial infarction or embolism.[125]

Granulocyte Colony-Stimulating Factor (G-CSF)

G-CSF is a cytokine growth factor approved by the FDA for bone marrow stimulation. Administration of G-CSF is known to mobilize stem cells from the bone marrow into the circulation and, like EPO, it has receptors in the brain.[121] Experimentally, G-CSF promotes new blood vessel formation in the IS penumbral area, and has anti-inflammatory, anti-excitotoxic, and neuroprotective properties that make it attractive for post-stroke rehabilitation.[126]

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Among stem cell researchers, two therapeutic approaches have emerged.[127] The first is to transplant exogenous stem cells; the second is to amplify endogenous stem cells. The first approach is currently limited by the logistics (and expense) of obtaining, expanding, and transplanting stem cell lines, not all of which may be safe to use.[116] G-CSF use supports the latter strategy.

In a small pilot RCT, Shyu et al gave G-CSF to acute stroke patients, then followed their neurological function and brain metabolic activity for 12 months.[128] Patients with recent MCA infarction (mild-to-moderate, per the mNIHSS) were randomized to receive the standard of care for acute stroke, then either G-CSF (n=7) (15 mcg/kg, subcutaneously for five consecutive days) or no additional treatment (n=3). Patients were discharged after one week, then followed up every 1-3 months for one year. Three of the G-CSF patients experienced headache, transient bone pain, and transient liver impairment, which resolved once treatment stopped. Leukocyte blood counts indicated G-CSF mobilized stem cells from the marrow to the bloodstream.

At 12 months, G-CSF patients demonstrated significantly greater neurological improvement than controls on four scoring scales employed (Figure 3); infarct sizes remained the same. PET imaging revealed the G-CSF group had significantly greater glucose metabolic activity in the penumbral zone than did the controls. This increased metabolic activity correlated significantly with increased neurological score on the European Stroke Scale Motor Subscale (EMS). The researchers noted that patients started on G-CSF within 24 hours after stroke showed better neurological improvement.

Conclusion

Time is brain. Stroke recovery ultimately depends on surviving the acute stroke. The priority in acute management must be to get the patient to a stroke care facility. FAST-MAG training of emergency personnel to administer IV magnesium in the ambulance is essential. An acute stroke management team should perform a complete neurological and cardiovascular stroke screening within 60 minutes. Eligibility for IV rtPA or intra-arterial thrombolysis should be determined as quickly as possible. With or without thrombolysis, neuroprotection can be implemented using albumin, magnesium, hypothermia, hyperbaric oxygen, or other interventions proven safe for the acute phase. Beyond the acute phase, safe nutrient neuroprotectants such as citicoline and GPC can be implemented.

Caution should be exercised using nutrient antioxidants during acute stroke. Antioxidants can become pro-oxidative in the presence of free iron, as when iron is detached from hemoglobin during hemorrhage; this may also occur to some degree during ischemic stroke. After several RCTs that failed to demonstrate cardiovascular protection of vitamin E,[129] Milman et al re-analyzed the data and concluded vitamin E may be beneficial only for individuals with significantly higher oxidative stress. They conducted a double-blind RCT with vitamin E (as d-alpha tocopherol, 400 IU/day) in diabetic patients with a specific genetic susceptibility to oxidative stress.[130] In this trial vitamin E offered significant protection against stroke, myocardial infarction, and cardiovascular death.

When the post-stroke patient is stabilized, the stage is set for rehabilitation and brain plasticity becomes the key to stroke recovery. As reviewed elsewhere,[99] the brain's processing machinery is sustained by intensive use under challenging conditions. Continuous, active interaction with environments demanding to the sensory, cognitive, and motor systems is necessary to maintain brain health. With the stroked brain stabilized, damage processes quenched, and zones of stunned cells reactivated through reperfusion, external stimulation can then be skillfully applied to literally remap the brain's cortex and fill in the blank functional zones.

A number of innovative physical and mental exercise approaches are available to help drive function-driven brain restoration (see Cramer[131] for a comprehensive review). They include constraint-induced movement therapy (CIMT), which is based on the idea of overcoming learned nonuse of the affected limb after stroke. CIMT involves restraining the good limb to force the bad limb into action. A recent clinical trial proved that two weeks of CIMT can produce gains that remained two years later.[132,133] The CIMT concept is also being extended to cognitive retraining.[131] Brain training ("neurobics") is showing promise. The best of the brain training exercises rely on the concept of brain plasticity.[134]

The future appears positive for stroke recovery based on the principles of brain plasticity. Research into stroke recovery will continue to overlap with other burgeoning fields of brain restoration, such as traumatic brain injury and spinal cord injury. A substantial target population for brain restoration exists, including stroke survivors, TBI survivors (including more than 320,000 from Iraq),[135] spinal cord injury survivors, and millions living with neurodegenerative diseases.

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