New hope for stroke patients: mobilization of endogenous stem cells

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Until recently, the inability of adult brain cells to regenerate after sustaining damage was an accepted scientific dogma. However, evidence has accumulated over the last decade that neurons and astrocytes can be generated from isolated cells of the adult mammalian central nervous system (CNS).[1] On the basis of this phenomenon of adult CNS plasticity, or neurogenesis, stem cell-based therapies have been developed for various CNS diseases, including stroke, traumatic brain injury and neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease. Two major lines of investigation have emerged advocating stem cell-based therapies, one focused on transplanting exogenous stem cells and the other on amplifying endogenous stem cells through neurogenesis.[2,3]

Stem cells have been proposed for use as highly efficacious donor cell grafts because of their ability to differentiate in vitro and in vivo into neurons, astrocytes and oligodendrocytes. This multipotency makes them excellent candidates for transplantation in the case of CNS diseases or injuries with complicated disruptions in neural circuitry, such as stroke, where more than one cell population is affected. Despite this great potential, an easily obtainable, abundant, safe and clinically proven source of stem cells has remain elusive.

Nonetheless, stem cell transplantation offers hope for stroke patients, especially for those who have missed the narrow 3-hour window for administration of tissue plasminogen activator. In 1998, we provided preclinical evidence that neuroteratocarcinoma (NT2N) cells, a clonal cell line considered neural progenitor cells, significantly attenuated motor and cognitive deficits when transplanted into adult rats 4 weeks after middle cerebral artery occlusion.[4] In that same year, the US Food and Drug Administration (FDA) approved a small-scale open-label clinical trial of NT2N cell transplantation in 12 stroke patients. The results of this study showed for the first time that cell transplantation for stroke is a potentially feasible and generally safe approach (on the basis of the results of a 2-year post-mortem study involving a stroke patient who underwent cell transplantation and who died of causes unrelated to the surgical procedure).[5] Because of the "cancerous" origin of NT2N cells, "safer" stem and progenitor cells have been explored as alternative graft sources.

Amplification of endogenous stem cells provides an alternative way of re-innervating the damaged brain and correcting neurologic impairments. Mobilizing host stem cells is less cumbersome than transplantation in that it avoids the logistical complexity associated with the use of embryonic as well as nonembryonic stem cells, including supply, surgical trauma, and the possibilities of graft rejection, uncontrolled graft cell proliferation and tumour formation, among others. The concept of stimulating the host microenvironment to achieve neurogenesis has a solid basis in science. For example, some disease states, like cerebral ischemia, have been shown to promote neurogenesis.[6] However, such disease-mediated neurogenesis is not sufficient to abrogate the cell death cascade and thereby requires supplemental intervention to realize CNS repair. To this end, laboratory studies have explored in vivo stimulation, including increased exercise to enhance neurogenesis.[7] Still others have examined therapeutic molecules that have a highly potent capacity for stem cell mobilization.

Among the many stem cell mobilization agents, granulocyte colony-stimulating factor (G-CSF) has received much attention among stem cell researchers. Administration of G-CSF (already an FDA-approved drug) mobilizes stem and progenitor cells from the bone marrow into the peripheral blood, from which they can "home" in to the brain and have a protective or restorative effect. In addition to its recently recognized anti-inflammatory and angiogenic properties, G-CSF has a neuroprotective effect: it reduced infarct size by 47% in a rat model of stroke due to occlusion of the middle cerebral artery when administered within 30 minutes of ischemic injury.[8] A subsequent study by the same research group[9] showed that G-CSF's "neuroprotection" takes the specific form of a direct trophic and protective effect on neurons: G-CSF exerts an anti-apoptotic effect on neurons possibly through its receptors, since these receptors are strongly up-regulated on neurons during cerebral ischemia. In addition, G-CSF stimulation of neural progenitor cells accompanying the observed functional recovery in cerebral ischemia models implicates neurogenesis as a mechanism underlying G-CSF's therapeutic benefits. These laboratory findings laid the groundwork for the research group to start a randomized and blinded phase II study of G-CSF for acute stroke patients in Germany, led by Dr. Wolf Rüdiger Schäbitz. Another stroke clinical trial of G-CSF is underway in the United Kingdom directed by Dr. Philip Bath.

In this issue (see page 927),[10] Shyu and colleagues explore the therapeutic potential of G-CSF therapy for ischemic stroke (i.e., acute cerebral infarction within the middle cerebral artery territory as verified by MRI upon hospital admission). Patients who scored between 9 and 20 in the National Institutes of Health Stroke Scale (NIHSS) were randomly assigned within 7 days of stroke onset to 5-day subcutaneous G-CSF therapy (15 μg/kg per day) ($n = 7$) or usual care ($n = 3$). Over 12-month follow-up, patients who received G-CSF displayed significantly

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greater improvement in neurologic function than control patients according to measurements using 4 clinical scales, including the NIHSS, European Stroke Scale, European Stroke Scale Motor Subscale and Barthel Index. The improvement became apparent by 6 months after stroke onset and was stable up to the end of the 12-month follow-up. Assessment of functional activity with PET at 12 months revealed significant improvement in cerebral uptake of fluorodeoxyglucose in the cortical areas surrounding the ischemic core in G-CSF patients compared with control patients. Moreover, a positive correlation between increased metabolic activity and motor stroke scale score was observed. No severe adverse effects were reported other than bone pain and headache, which were tolerated by the patients. This small pilot trial is the first clinical study reporting on the safety and feasibility of G-CSF therapy in stroke patients and should be critically analyzed with the 2 European clinical trials. As Shyu and colleagues acknowledge, the small sample precludes any solid conclusions regarding efficacy. Thus, the preliminary evidence in this phase I study is tenuous at best. Efficacy and further confirmatory safety data will need to come from larger phase II studies that are randomized and blinded.

Current treatment for stroke, particularly thrombolytic therapy with tissue plasminogen activator, is problematic because of its short window of efficacy, and new treatment modalities are therefore needed, particularly those with a longer window. The earliest administration of G-CSF in the study by Shyu and colleagues was at day one after stroke onset. Such a wider therapeutic window would be a significant clinical advance since patients often do not reach hospital — nor is the disease often diagnosed — until later than 3 hours after onset. However, the timing of G-CSF administration is likely to influence its neuroprotective effects. Indeed, in this study, patients who received G-CSF at day one after stroke onset exhibited much greater improvement in neurologic function than those who received it later than day one. Factors that need to be optimized to enhance G-CSF's therapeutic benefits include the integrity of the blood–brain barrier so that mobilized stem cells can penetrate the brain and home into the stroke site, and up-regulation of chemokines and their receptors, cytokines, and adhesion molecules, which may direct the migration of the stem cells to the stroke region. Since G-CSF may act primarily on neurons, it is likely that the earlier the treatment, the more potent the neuroprotective effect. The optimal dose of G-CSF also needs to be examined, since the study by Shyu and associates used 15 μg/kg per day and the European trials include higher doses of 180 μg/kg per day.

The visualization of stem cells mobilized by G-CSF would provide a direct measure of their participation in the neurologic improvement observed in this study. For stable functional recovery, it is likely that the stem cells mobilized by G-CSF need to remain viable in the post-ischemic brain, at least within the partially viable ischemic penumbra. A noninvasive strategy to monitor their survival and migration over time will provide insight into the mechanisms underlying the observed behavioural recovery of stroke patients. Assessment of whether stem cells retain their stem cell marker phenotypic expression or change that expression (e.g., to that of neuronal or endothelial cells) should be pursued in the long term. Alternatively, determining whether G-CSF stimulates secretion of growth factors or neurotrophic factors or both may reveal additional mechanisms, as we and others have recently demonstrated following stem or progenitor cell transplantation in the setting of acute ischemic stroke.[11,12] This evaluation can be easily performed by collecting peripheral blood samples from G-CSF patients. The concept that mobilized cells potentially contribute neurotrophic, proliferative, chemoattractant and migratory factors abetting repair is intriguing but remains to be proven.

The findings of Shyu and colleagues are in concert with growing optimism regarding the future of stem cell therapy for stroke. There is currently an active effort to pursue testing stem cell therapy in nonhuman primate stroke models before proceeding with clinical trials. In the end, critical analyses, well-designed preclinical studies and limited clinical trials of the safety, toxicity, optimal drug dosage, route and timing of delivery post-stroke will ultimately determine whether or not we are ready to advance G-CSF therapy into definitive large-scale clinical application for stroke.

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REFERENCES

1. Reynolds BA, Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* 1992;255:1707-10.
2. Hess DC, Hill WD, Carroll JE, et al. Do bone marrow cells generate neurons? *Arch Neurol* 2004;61:483-5.
3. Haas S, Weidner N, Winkler J. Adult stem cell therapy in stroke. *Curr Opin Neurol* 2005;18:59-64.
4. Nishino H, Borlongan CV. Restoration of function by neural transplantation in the ischemic brain. *Prog Brain Res* 2000;127:461-76.
5. Nelson PT, Kondziolka D, Wechsler L, et al. Clonal human (hNT) neuron grafts for stroke therapy: neuropathology in a patient 27 months after implantation. *Am J Pathol* 2002;160:1201-6.
6. Sun Y, Jin K, Xie L, et al. VEGF-induced neuroprotection, neurogenesis, and angiogenesis after focal cerebral ischemia. *J Clin Invest* 2003;111:1843-51.
7. Van Praag H, Kempermann G, Gage FH. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat Neurosci* 1999;2:266-70.
8. Schabitz WR, Kollmar R, Schwaninger M, et al. Neuroprotective effect of granulocyte colony-stimulating factor after focal cerebral ischemia. *Stroke* 2003;34:745-51.
9. Schneider A, Kruger C, Steigleder T, et al. The hematopoietic factor G-CSF is a neuronal ligand that counteracts programmed cell death and drives neurogenesis. *J Clin Invest* 2005;115:2083-98.
10. Shyu WC, Lin SZ, Lee CC, et al. Granulocyte colony-stimulating factor for acute ischemic stroke: a randomized controlled trial. *CMAJ* 2006;174(7):927-33.
11. Borlongan CV, Skinner SJ, Geaney M, et al. Intracerebral transplantation of porcine choroid plexus provides structural and functional neuroprotection in a rodent model of stroke. *Stroke* 2004;35:2206-10.
12. Chen X, Li Y, Wang L, et al. Ischemic rat brain extracts induce human marrow stromal cell growth factor production. *Neuropathology* 2002; 22:275-9.

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