Intravascular cell replacement therapy for stroke

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The use of stem cell transplantation to restore neurological function after stroke is being recognized as a potential novel therapy. Before stem cell transplantation can become widely applicable, however, questions remain about the optimal site of delivery and timing of transplantation. In particular, there seems to be increasing evidence that intravascular cell delivery after stroke is a viable alternative to intracerebral transplantation. In this review, the authors focus on the intravascular delivery of stem cells for stroke treatment with an emphasis on timing, transendothelial migration and possible mechanisms leading to neuroprotection, angiogenesis, immunomodulation, and neural plasticity. They also review current concepts of in vivo imaging and tracking of stem cells after stroke.

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KEY WORDS • intravascular cell delivery • neuroprotection • regenerative medicine • stem cells • stroke

Abbreviations used in this paper: BDNF = brain-derived neurotrophic factor; BMSC = bone marrow stem cell; CNS = central nervous system; EBST = elevated body swing test; FACS = fluorescence activated cell sorting; GDNF = glial-derived neurotrophic factor; hNT = human teratocarcinoma-derived cell line; HUCBC = human umbilical cord blood cell; ICAM-1 = intercellular adhesion molecule–1; MR = magnetic resonance; MSC = mesenchymal stem cell; NPC = neural progenitor cell; NSC = neural stem cell; PET = positron emission tomography; SDF-1 = stromal-derived factor–1; SPIO = superparamagnetic iron oxide; USPIO = ultrasmall SPIO; VCAM-1 = vascular cell adhesion molecule–1; VEGF = vascular endothelial growth factor.

STROKE remains the number 1 cause of disability and the 3rd leading cause of death among Americans each year. The incidence of stroke in the US is 730,000 new or recurrent cases of stroke/year with an estimated 6,000,000 stroke survivors. According to the National Institutes of Health, the estimated direct and indirect costs related to stroke for 2007 total $62.7 billion.

Despite the significant socioeconomic burden attributed to stroke, there are no therapeutic approaches currently available besides thrombolytic treatments, which have a narrow time window and limited availability. However, stem cell–related treatments represent a novel and promising therapeutic avenue. Preclinical data regarding stem cell therapy for the treatment of stroke are impressive and have raised hopes that such new therapies can be used to treat the sequelae of a devastating stroke. Furthermore, stem cell transplantation therapy is already a well-established treatment modality for patients with hematopoietic and lymphoid cancers and some autoimmune disorders.

Three human trials in which researchers assessed the utility of stem cell therapies for stroke have been performed to date. All 3 trials included patients who were treated at least 6 months after their strokes (between 6 months and 4.5 years, between 1 and 6 years, and between 1.5 and 10 years). In 2 of the trials an hNT was used, and in the 3rd trial porcine-derived NPCs were utilized. All 3 trials involved stereotactic transplantation of the respective cells into the border zone of the stroke. One patient died of an unrelated cause, and an autopsy was performed. The authors describe a 1-cm3 gliotic area medial to the infarct consistent with the site of the grafted hNT neurons. Viable neurons were detected using immunohistochemistry and fluorescence in situ hybridization. Although cell transplantation turned out to be safe and well tolerated, the limited data from available preliminary studies in patients indicate that further research is mandatory to improve the procedure’s clinical efficacy.

These human trials raised several points of discussion with regard to optimal transplantation conditions, including what type, number, and concentration of cells ought to be transplanted; how long after the stroke should the surgeon wait before transplantation; and what is the best site of transplantation. In addition to these procedure-related issues, mechanistic concerns have been raised. In particular, does the stereotactic transplantation technique allow transplanted cells to reach all of the appropriate sites of action on the microscopic scale? Numerous studies have revealed extensive migration of transplanted stem cells.
after inflammation and stroke in the rodent brain.\textsuperscript{31,33,37,58} Data from several studies have also enhanced our understanding of these chemotactic mechanisms, including key chemoattractant factors, such as SDF-1\textsuperscript{16} and monocyte chemoattractant protein–1.\textsuperscript{87} However, in contrast to the rodent brain, only very limited migration has been observed in the adult human brain.\textsuperscript{61} As a result, cellular delivery techniques that rely on cellular migration less than stereotactic transplantation are desirable for clinical use.

One such technique is the intravascular delivery of stem cells, which has the potential for a wider distribution of the cells as compared with stereotactic transplantation, thereby relying less on cellular migration. Furthermore, this technique has the added clinical benefit of being less invasive than stereotactic transplantation.

In this review, we focus on the intravascular delivery of stem cells as a treatment for stroke. We discuss the mechanisms thought to be involved in the transendothelial migration of stem cells and the related timing of transplantation, which seems to be crucial for this route of cell delivery. Finally, we discuss potential mechanisms of action through which the injected stem cells improve functional recovery after a stroke and how the migration of the transplanted cells could be tracked using in vivo imaging.

### Route of Stem Cell Delivery

Reports have indicated that after stereotactic intraparenchymal,\textsuperscript{17,46} intracerebroventricular,\textsuperscript{60} intravenous,\textsuperscript{12,13,89} and intraarterial transplantation,\textsuperscript{32,53,73,74} stem cells can home to sites of injury in the CNS and induce functional recovery. Of these various transplantation techniques, those that depend on intravascular delivery of stem cells for stroke are particularly attractive. In addition to its noninvasive nature, intravascular delivery may allow stem cells to have a superior interaction with injured tissue. A comparative study revealed that direct intracerebral transplantations resulted in the largest number of cells at the lesion site, followed by intracerebroventricular and intravenous transplantations.\textsuperscript{44} However, researchers in that study only assessed the absolute number of cells in the perilesional area and took no account of whether these cells were therapeutically distributing to all injured areas of brain parenchyma on a microscopic level. Many believe that intravascular delivery of stem cells may lead to a wider distribution of cells around the lesioned area as compared with focal perilesional transplants, thereby leading to superior stem cell–injured tissue interactions.

Several groups have studied the mechanisms by which intravenous transplantation leads to a wider distribution. The cells travel in the bloodstream and follow a chemotactic gradient generated by inflammation in the injured brain.\textsuperscript{85,86} Unfortunately, intravenously delivered cells pass through the systemic and pulmonary circulation systems and home to organs as well, which significantly reduces cell homing to the injured brain.\textsuperscript{8} Li and colleagues\textsuperscript{52} intravenously injected 3 × 10\textsuperscript{6} human MSCs into rats 24 hours after stroke and showed that only 4% of the cells entered the brain. Similarly, a study by Chu and colleagues\textsuperscript{18} demonstrated that after an intravenous injection of 5 × 10\textsuperscript{6} immortalized human NPCs following stroke, only a small number of cells entered the brain. Interestingly, the number of cells entering the brain increased over time and peaked at Day 21 poststroke. At Day 56, 60% of these surviving cells differentiated into glia, and 20% into neurons. Despite the fact that the number of cells entering the brain was limited, functional recovery was enhanced by intravenous delivery. The intravenous transplantation of a minimum of 10\textsuperscript{4} HUCBCs reduced hyperactivity, whereas a minimum of 10\textsuperscript{5} HUCBCs improved recovery in the step test and a minimum of 10\textsuperscript{6} HUCBCs improved recovery in the EBST.\textsuperscript{82}

The main concern regarding intravenous stem cell injections is the whole-body distribution of cells. Pluchino and associates\textsuperscript{66} found that, after the intravenous injection of NPCs in an experimental autoimmune encephalomyelitis mouse model, only 3% of the cells would home to the CNS, whereas a majority of the cells would be found in the kidney, liver, and spleen. Therefore alternative routes of intravascular delivery have been explored.

Another route of intravascular delivery is intraarterial, which would circumvent body circulation. The first pass of stem cells injected into the carotid artery would be the brain. Authors of a few studies have explored this route of delivery and have demonstrated functional recovery after stroke\textsuperscript{6,17,18} and traumatic brain injury.\textsuperscript{11,12} Shen and colleagues\textsuperscript{74} injected 2 × 10\textsuperscript{6} donor rat BMSCs into the internal carotid artery of rats 24 hours poststroke and successfully induced functional recovery. In another study, the same group injected 2 × 10\textsuperscript{6} donor rat BMSCs into rats 24 hours after stroke and observed that injected cells localized around the infarction area in the brain and very few were found in the heart, lungs, liver, spleen, and kidney.\textsuperscript{74} Similarly, Li and colleagues\textsuperscript{53} injected 2 × 10\textsuperscript{6} donor rat BMSCs into the internal carotid artery of rats and found that nearly 21% of these cells entered the brain and induced functional recovery.

Our group has also demonstrated the benefits of intracarotid artery injection. We found that the intracarotid artery injection of 3 × 10\textsuperscript{6} FACS-sorted CD49+ immortalized mouse NPCs after stroke lead to high numbers of NPCs entering the brain and improving functional recovery.\textsuperscript{62} High cell concentrations (up to 1300 cells/mm\textsuperscript{3}) in the areas affected by stroke, including the striatum, cortex, and hippocampus, were achieved with intracarotid artery injections (Fig. 1). These cell concentrations exceed those reported in studies with intravenous injections (74 cells/mm\textsuperscript{3}).\textsuperscript{56}

The debate over the best delivery route is further complicated by the fact that there is still a great deal of controversy concerning the mechanism by which stem cells lead to enhanced functional recovery in patients who have experienced stroke. The 2 most discussed mechanisms are as follows: 1) cellular replacement, by way of the functional integration of stem cells; and 2) secretion of neurotrophic and angiogenic factors. If the mechanism of recovery is cellular replacement, then transendothelial migration is necessary and the methods that allow the highest concentrations of stem cells in the injured brain areas ought to be pursued; however, there is significant evidence that stem cells may provide their benefits by secreting various neuroprotective factors. In fact, most of the studies involving intravenous cell delivery either failed to demonstrate, or at best showed only a very small proportion of, the injected cells entering the injured brain.\textsuperscript{2,12} Despite this poor trans-
endothelial migration, intravenous cell delivery still leads to enhanced functional recovery. Borlongan and associates showed that CNS entry of peripherally injected umbilical cord blood cells was not required for neuroprotection in stroke. Despite the addition of mannitol to open the blood–brain barrier, no cells were found in the brains of animals that had experienced stroke; however, significantly elevated levels of GDNF, a potent neuroprotective factor, were detected. Chu and colleagues and Li et al. found only small numbers of cells entering the CNS, but nevertheless found significant behavioral recovery after cell treatment. The same was true in a nonstroke model in which only 3% of the injected cells entered the CNS.

In summary, the best route of human stem cell delivery has not been determined, but the intravascular route is particularly attractive because of its ease of administration, minimal invasiveness, and potential for widespread cell distribution together with widespread secretion of neuroprotective, proangiogenic, and immunomodulatory factors. Intuitively, the intraarterial route of delivery seems better than the intravenous, given that injected cells first pass the target organ—that is, the brain—prior to being redistributed in the systemic circulation.

**Timing of Transplantation**

The poststroke brain is characterized by several unique conditions that vary with time, including increased excitotoxicity, perinfarction depolarization, the presence of radical oxygen species, inflammation, and cell death. Unoubtedly, the fate and function of transplanted cells after stroke will depend on any or all of these alterations, and the optimal time of transplantation after stroke is unknown. The timing of transplantation depends mainly on the goal of treatment, for example, neuroprotection, which should happen early after the insult, or neuroregeneration/cell replacement, which can be done once a lesion has stabilized. We can envision a future in which we will rely on multimodal stem cell treatment, depending on a combination of early and late administrations of different cell types. For the purpose of this review, however, we will concentrate on the early intravascular delivery of stem cells after stroke.

**Early Intravascular Cell Delivery**

Because the brain’s pathological environment goes through several changes after a stroke, the timing of poststroke stem cell injection determines our ability to take maximal advantage of these endogenous changes, which may favor transendothelial migration. In animal models with a neuroinflammatory component such as stroke, traumatic brain injury, spinal cord injury, and multiple sclerosis, therapeutic somatic stem cells (for example, BMSCs, umbilical cord blood stem cells, MSCs, and NPCs) target inflamed CNS areas where they persist for months and promote recovery through neuroprotective mechanisms. It is thought that the process of transendothelial migration of somatic stem cells may be regulated in a manner similar to that of inflammatory cells. As early as 30 minutes after stroke, the infiltration of leukocytes, both

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**Fig. 1.** Confocal laser scanning microscopy images revealing numerous cells in the stroke border zone and the hippocampus ipsilateral to the stroke (A). Inset shows doublecortin-bromodeoxyuridine–labeled cells. The VCAM-1 (arrows) is highly expressed in the stroke-affected hemisphere 48 hours after stroke (B). DCX = doublecortin; BrdU = bromodeoxyuridine; DAPI = 4′6-diamidino-2-phenylindole.
### TABLE 1

**Summary of preclinical studies addressing cell transplantation after stroke**

<table>
<thead>
<tr>
<th>Authors &amp; Year</th>
<th>Stroke Model</th>
<th>Cell Type/ Transplant Method</th>
<th>No. of Cells</th>
<th>Transplant Timing</th>
<th>No. of Animals</th>
<th>Immuno-suppression</th>
<th>Effect on Lesion Size</th>
<th>Survival &amp; Migration to Lesion</th>
<th>Phenotype</th>
<th>Functional Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chen et al., 2001</td>
<td>MCAO</td>
<td>HUCBCs/IV</td>
<td>$3 \times 10^6$</td>
<td>24 hrs or 1 wk</td>
<td>11</td>
<td>none</td>
<td>most cells in IBZ; 24 hrs pg killed at 2 wks; 2% NeuN, 3% MAP2, 6% GFAP, 8% vWF</td>
<td>24 hrs pg killed at 2 wks; 2% NeuN, 3% MAP2, 6% GFAP, 8% vWF</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Li et al., 2001</td>
<td>MCAO</td>
<td>rat BMSCs/ ICA</td>
<td>$2 \times 10^6$</td>
<td>24 hrs</td>
<td>16</td>
<td>none</td>
<td>21% of cells in brain</td>
<td>10% glia, 1% neurons</td>
<td>ND</td>
<td>improvement on adhesive-dot-removal &amp; mNSS tests</td>
</tr>
<tr>
<td>Li et al., 2002</td>
<td>MCAO</td>
<td>human MSCs/ IV</td>
<td>$3 \times 10^6$</td>
<td>24 hrs</td>
<td>12</td>
<td>none, but less apoptosis in IBZ</td>
<td>4% of cells found in brain; 60% of these in IBZ at Day 14</td>
<td>1% NeuN, 1% MAP2, 5% GFAP, 2% vWF</td>
<td>ND</td>
<td>recovery by 2 wks on sticky tape test &amp; NSS; increase in BDNF; NGF found in ischemic hemisphere</td>
</tr>
<tr>
<td>Chen et al., 2003†</td>
<td>MCAO</td>
<td>human MSCs/ IV</td>
<td>$10^6$</td>
<td>24 hrs</td>
<td>12</td>
<td>none</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Chu et al., 2003</td>
<td>CCAO + hypotension</td>
<td>HB1.F3/IV or ITV</td>
<td>$5 \times 10^6$‡</td>
<td>24 hrs</td>
<td>24</td>
<td>CSA daily, starting 1 day before grafting</td>
<td>ND</td>
<td>same no. of cells in brain for IV vs ITV</td>
<td>65% glia, 13% neurons at Day 56</td>
<td>ND</td>
</tr>
<tr>
<td>Willing et al., 2003§</td>
<td>MCAO</td>
<td>HUCBCs/IC</td>
<td>IC: $2.5 \times 10^5$; IV: $10^6$</td>
<td>24 hrs</td>
<td>12</td>
<td>CSA daily, starting the day of grafting</td>
<td>ND</td>
<td>0% at 2 mos</td>
<td>ND</td>
<td>IC &amp; IV led to same level of improvement on activity &amp; passive avoidance tests; IV led to better improvement on step test; no effect on rotarod test or EBST</td>
</tr>
<tr>
<td>Chu et al., 2004</td>
<td>MCAO</td>
<td>HB1.F3/IV</td>
<td>$5 \times 10^6$</td>
<td>24 hrs</td>
<td>103</td>
<td>none</td>
<td>no. of cells in brain increased over time, peak at Day 21</td>
<td>50% glia, 0% neurons at Day 14, 60% glia, 20% neurons by Day 56</td>
<td>ND</td>
<td>improvement on rotarod test &amp; EMST</td>
</tr>
<tr>
<td>Vendrame et al., 2004††</td>
<td>MCAO</td>
<td>HUCBCs/IV</td>
<td>$10^6$, $10^{6.5}$, $10^7$, or $3 \times 5 \times 10^7$</td>
<td>24 hrs</td>
<td>28</td>
<td>CSA daily, starting the day of grafting</td>
<td>decreased at higher cell doses</td>
<td>small no. of cells in brain, mostly around infarct in vasculature</td>
<td>ND</td>
<td>all doses (except $10^6$) reduced hyperactivity at 2 and 4 wks; $10^6$, $3 \times 5 \times 10^7$ improved on EBST at 4 wks; $10^7$, 1 &amp; $3 \times 5 \times 10^7$ improved on step test at 4 wks</td>
</tr>
<tr>
<td>Chu et al., 2005</td>
<td>MCAO</td>
<td>HB1.F3 ± VEGF/IV</td>
<td>$5 \times 10^6$</td>
<td>24 hrs</td>
<td>24</td>
<td>none</td>
<td>decreased, more so in NSC + VEGF pg</td>
<td>ND</td>
<td>cells + VEGF recovery greater than for either alone on rotarod test &amp; MLPT</td>
<td></td>
</tr>
<tr>
<td>Shen et al., 2006</td>
<td>MCAO</td>
<td>rat BMSCs/ ICA</td>
<td>$2 \times 10^6$</td>
<td>24 hrs</td>
<td>9</td>
<td>none</td>
<td>ND</td>
<td>ND</td>
<td>improvement on adhesive-dot-removal, mNSS, &amp; corner tests</td>
<td></td>
</tr>
<tr>
<td>Guzman et al., 2007**</td>
<td>MCAO</td>
<td>mouse C17.2/ ICA</td>
<td>$3 \times 10^5$</td>
<td>48 hrs</td>
<td>18</td>
<td>none</td>
<td>cells in perifarct area, hippocampus, &amp; SVZ</td>
<td>nestin, doublecortin, β-tubulin, GFAP Iba-1</td>
<td>ND</td>
<td>recovery on rotarod test</td>
</tr>
<tr>
<td>Shen et al., 2007</td>
<td>MCAO</td>
<td>rat BMSCs/ ICA</td>
<td>$2 \times 10^6$</td>
<td>24 hrs</td>
<td>8</td>
<td>none</td>
<td>none, but thinner glial scar wall seen</td>
<td>some cells in brain &amp; very few in heart, lung, liver, spleen, &amp; kidney</td>
<td>ND</td>
<td>improvement on adhesive-dot-removal &amp; mNSS tests</td>
</tr>
</tbody>
</table>

* C17.2 = immortalized mouse NPCs; CCAO = common carotid artery occlusion; CSA = cyclosporin A; GFAP = glial fibrillary acidic protein, a marker of astrocytes and neural progenitors; HB1.F3 = immortalized NPCs (14 wks gestation); IA = intraarterial; IBZ = ischemic boundary zone; IC = intracerebral; ipsi = ipsilateral; ITV = intraventricular; IV = intravenous; MCAO = middle cerebral artery occlusion, primarily damaging the striatum and some cortex; MLPT = modified limb placement test; mNSS = modified neurological severity score; ND = not determined; nestin = marker for NPCs, MSCs, and young endothelial cells; NeuN, MAP2 = neuronal markers; NGF = nerve growth factor; NSC = neural progenitors; pg = postgrafting; SVZ = subventricular zone; vWF = Von Willebrand factor, an endothelial cell marker. † Reference 14. ‡ For both routes. § Reference 88. ††Six animals for each group. †† Six animals for each number of cells, except that 4 animals were used for $5 \times 10^6$ cells. ** Reference 32.
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...polymorphonuclear leukocytes and monocytes/macrophages, can be observed. The temporal expression profile of adhesion molecules, cytokines (interleukin-1, interleukin-6, tumor necrosis factor–α, and transforming growth factor–β) and chemokines (cytokine-induced neutrophil chemoattractant, SDF-1, monocyte chemoattractant protein–1) has been shown to reach a peak level 24 hours after experimental stroke. At the bedside, soluble VCAM-1 concentration in plasma is increased in patients with acute stroke. Interstitial adhesion molecule–1 levels have been elevated as early as 4 hours after stroke with sustained levels for up to 1 week. Monocyte chemoattractant protein–1, a key chemoattractant factor for the recruitment of circulating peripheral cells to the stroke area and an important factor for stem cell migration, is upregulated 3 days after stroke and then returns to baseline after 1 week. Similarly, SDF-1 is known to be a potent chemoattractant for inflammatory cells as well as stem cells (including BMSCs and NSCs) and is expressed early after stroke. Anatomically, adhesion molecule upregulation as well as chemokine expression has been shown to be highest in the stroke-affected penumbra region. Blocking the different pathways of chemotraction and cell adhesion in stroke-affected rodents reduced the number of infiltrating inflammatory cells. In mice lacking ICAM-1, a significant reduction in inflammatory cellular infiltrate and a reduction in lesion size was noted. Treatment with anti–ICAM-1 antibodies was a successful neuroprotective means of reducing lesion size and apoptosis in experimental stroke. However, a clinical trial exploring the feasibility of using an ICAM-1 blocking antibody failed to demonstrate any beneficial effects in the patients.

There is some evidence that intravascularly administered stem cells undergo the same process as inflammatory cells, including chemotraction, adhesion, and transendothelial migration after stroke, potentially making this route an ideal way of cell delivery. Several studies have demonstrated that NPCS express surface integrins such as CXCR4, CCR2, CD49d. In their model of CNS inflammatory disease, Pluchino et al. demonstrated that blocking the cell surface integrin CD49d (ligand to VCAM-1) reduced the number of NSCs homing to the brain by 60%. Similarly, our group has also shown the importance of CD49d, as we found that enriching NSCs for CD49d expression by FACS significantly enhanced transendothelial migration and homing to the stroke site after intracarotid artery cell delivery.

Based on these findings, homing of somatic stem cells delivered to the intravascular compartment to the site of injury might be mediated by several processes of the poststroke inflammatory response. The early onset of these processes and their close anatomical relation to the stroke border zone make them an ideal "natural" cell delivery mechanism. Hence, the success of early intravascular cell therapy might be guided by the inflammatory response of the injured brain.

Late Intraparenchymal Cell Transplantation

In contrast to the acute intravascular cell treatment, the intraparenchymal approach has been hindered by poor outcomes if the stem cells are transplanted too early after stroke. Excitotoxicity, oxidative stress, and inflammation postischemia make the ischemic brain a hostile environment for intracerebrally transplanted cells. In fact, we have found a negative correlation between graft survival and inflammation as measured by IB4 immunoreactive cells. Additionally, we demonstrated that human NSCs transplanted too close or into the stroke area have very limited survival at 7 days after stroke. Similar to our findings, Grabowski et al. found that transplanting cells 3 weeks after stroke, when there is a significant decrease in inflammation, led to greater graft survival than transplanting 5–7 days after stroke.

Taken together, early intravascular cell therapy might benefit from the processes tied to poststroke inflammation but might be detrimental to cells directly transplanted intraparenchymally. Therefore, intraparenchymal cell replacement therapy might be useful as a second-line or delayed stem cell treatment strategy for stroke. There are no data supporting late intravascular cell delivery after stroke, and further investigation will be needed to explore this possibility.

Potential Mechanism Involved in Stem Cell–Mediated Recovery After Stroke

Initial transplantation studies were focused on the potential of NSCs to replace lost circuitry. Transplanted NPCs in a rat model of global ischemia and hNT neurons in a model of traumatic brain injury have been reported to express synaptic proteins posttransplantation. However, only limited evidence demonstrates that transplanted cells are able to sustain CNS repair through massive cell replacement, especially to the extent that might be required after stroke. Regardless of the characteristics of the experimental disease, functional recovery achieved through NPC transplantation does not correlate with absolute numbers of transplant-derived, newly generated, terminally differentiated neural cells. The propensity for maintaining an undifferentiated phenotype within host tissue suggests that transplanted cells may act through a variety of alternative mechanisms. We will focus on the neuroprotective, angiogenic, and immunomodulatory effects of transplanted NPCs.

Stem Cell–Induced Neuroprotection

Transplanted stem cells can provide neuroprotection and reduce host cell death in the poststroke brain. Most authors have reported functional recovery and a reduction in lesion size when cells are transplanted within the first 24–48 hours after stroke. The short timeframe in which NPCS affect recovery cannot be explained by the regeneration of new neurons and synapses, suggesting an important role for neuroprotection in enhancing recovery. In fact, NPCS are known to exert direct neuroprotection through the neutralization of free radicals, inflammatory cytokines, excitotoxins, lipases, peroxidases, and other toxic metabolites that are released following an ischemic event.
The neuroprotective effects of transplanted NPCs are usually accompanied by increased in vivo bioavailability of main neurotrophins such as nerve growth factor, BDNF, ciliary neurotrophic factor, VEGF, fibroblast growth factor, and GDNF. Intriguingly, Borlongan et al. demonstrated that cells genetically engineered to produce GDNF have a significant neuroprotective effect despite not actually entering the brain parenchyma after intravascular delivery. Alternatively, one could argue that the cells did adhere to the endothelium in the affected brain area and exerted their effect through the secretion of GDNF but did not eventually engraft.

A different aspect of neuroprotection would be an effect on endogenous neurogenesis after stroke. Endogenous neurogenesis is increased after a stroke. Its exact function has yet to be determined, but it may signify a natural repair mechanism of the brain, which could be enhanced by transplanted cells. There is precedence for this effect with cord blood cells and BMSCs, but to date there are no reports on the effect of grafted NPCs on endogenous neurogenesis.

Stem Cell–Induced Angiogenesis

Transplanted NPCs can also enhance endogenous angiogenesis. Increased vascularization in the penumbra within a few days after stroke is associated with spontaneous functional recovery. As early as 3 days after ischemic injury, new blood vessels are observed in the stroke-affected penumbra, and growth continues to increase for at least 21 days. Transplanted cell–induced blood vessel formation has been reported with BMSCs, NSCs, and cells from human cord blood or peripheral blood. The ability of transplanted cells to increase endogenous levels of angiogenic factors (such as the aforementioned VEGF, fibroblast growth factor, and BDNF) and chemokine factors (for example, SDF-1) induces the proliferation of existing vascular endothelial cells (angiogenesis) and mobilization and homing of endogenous endothelial progenitors (vasculogenesis). Yano and associates have demonstrated that encapsulated cells producing VEGF significantly increase endogenous angiogenesis and improve functional outcome after stroke. The direct injection of recombinant SDF-1 into the stroke-affected rat brain resulted in the increased recruitment of BMSCs and increased vascular density in the ischemic brain. A similar effect was achieved by treatment of stroke-affected rats with granulocyte colony-stimulating factor. We found a significantly higher density of lectin-positive blood vessels in the stroke border zone in animals injected with NSCs into the carotid artery as compared with vehicle-injected animals.

Questions remain about whether stem cell–induced angiogenesis after stroke is sustained or whether it is a transient mechanism leading to neuroprotection. Further studies are needed to elucidate the ideal cells, the ideal time point of injection, and the mechanisms other than VEGF secretion that are involved in this process.

Stem Cell–Induced Immunomodulation

In addition to neuroprotection and enhancement of angiogenesis, transplanted NPCs can also decrease postischemic inflammatory damage. A landmark study highlights the remarkable ability of transplanted NPCs to promote neuroprotection through an immunomodulatory strategy. Once within inflamed CNS areas, systemically injected NPCs persist around the perivascular space where reactive astrocytes, inflamed endothelial cells, and blood-borne infiltrating T cells crosstalk. Pluchino and associates have shown that adult NPCs can promote neuroprotection by releasing antiinflammatory chemokines and by expressing immunomodulatory molecules (for example, Fasl, Apo3Li, and TRAIL). In a mouse model of multiple sclerosis (experimental autoimmune encephalomyelitis), NPCs promote apoptosis of effector cells expressing death receptors (for example, encephalitogenic Th1 cells). In animal models of stroke, decreased infiltration of mononuclear cells have been demonstrated at the lesion borders of ischemic areas in the CNS where NPCs accumulate.

Working under the paradigm of bidirectional communication between transplanted cells and the inflammatory ischemic microenvironment, one group has attempted to combine NPC-based and immunotherapy-based approaches in spinal cord injury. Ziv and colleagues described a 2-stage process of repair. In the first stage, myelin-specific T cells induce transplanted NPC migration to the injury site and also activate microglia to adopt a neuroprotective phenotype; in the second stage, NPC interaction with T cells and microglia leads to better tissue preservation, increased endogenous neurogenesis, and improved functional recovery. Although basic differences between the postischemic brain and the postinjury spinal cord might preclude the translation of this approach to stroke, the common themes of a limited therapeutic window and coopting immune cells to promote a more neurogenic microenvironment beckon a closer look. Indeed, studies by these same authors have revealed how modulation and/or suppression of inflammation after stroke can promote neuroprotection and neurogenesis. This fascinating cross-talk between neural and immune cells reveals additional therapeutic opportunities for attenuating postischemic inflammatory damage and providing a more hospitable environment for the persistence of both endogenous and transplanted NPCs.
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Neural stem cells engineered to overexpress thrombospondins to enhance posts ischemic synaptogenesis might be an interesting path to explore.

Possible Ways to Monitor Cell Treatment

The clinical application of cell transplantation therapy for stroke requires a means of noninvasively monitoring these cells in patients. Several imaging modalities, including MR imaging, PET, and in vivo fluorescence microscopy, have been used to track stem cells in vivo. Of these methods, however, only MR imaging and PET are practicable for clinical use.

Magnetic resonance imaging is a high spatial resolution method for tracking cells in real time. Cells are preloaded with molecules that substantially alter proton resonance, such as SPIO and USPIO particles. In vivo, SPIO-labeled cells appear as hypointense areas in tissues, because of a susceptibility artifact most pronounced in iron-sensitive T2- and T2*-weighted images. With high-resolution MR imaging, even single mammalian cells have been depicted after SPIO labeling. Several studies have shown the ability of MR imaging to longitudinally track transplanted iron-labeled cells in different animal models, including stroke. Using clinical grade human fetal-derived NSCs, we demonstrated that the transfection of cells with protamine sulfate and SPIO did not alter cell proliferation, differentiation, migration, or electrophysiological characteristics. One human trial for traumatic brain injury has demonstrated the feasibility of tracking SPIO-labeled stem cells in humans. However, one of the drawbacks of SPIO labeling of stem cells is the dilution of SPIO as the cells proliferate; therefore, new methods utilizing reporter genes are being explored. The most promising method is the transfusion of stem cells with a PET reporter gene. Compared with MR imaging, the detection threshold of PET is 7 log orders more sensitive (10^4 vs 10^11 mol/L, respectively). The currently described PET method for cell tracking uses a trifusion reporter construct encoding for a bioluminescence synthetic Renilla luciferase (hRL) reporter gene, a reporter gene encoding the monomeric red fluorescence protein (MRFP1), and a mutant herpes simplex virus Type 1 sr39 thymidine kinase (HSV1-truncated sr39tk [ttk]), a PET reporter gene. Successful transfection of tumor cell lines and longitudinal in vivo imaging up to 50 days posttransplantation were demonstrated using combined PET and bioluminescence imaging. Molecular PET imaging of reporter gene expression has already been successfully translated into clinical trials involving patients with recurrent glioblastoma multiforme; however, PET has low spatial resolution and a lack of anatomical information compared with MR imaging. Future imaging modalities may involve a combination of the high spatial resolution and excellent anatomical detail of MR imaging with the high sensitivity of PET. An alternative direction for stem cell imaging is the development of MR reporter genes. In their study, Genove et al. used a replication-defective adenovirus to introduce an MR imaging reporter gene encoding metalloproteins from the ferritin family into specific tissues of a living patient. The vector-encoded reporter is made superparamagnetic as the cell sequesters endogenous iron from the organism. One great advantage of this technique is that no external contrast agent needs to be administered for imaging. However, the application of MR reporter genes for in vivo studies has been extremely limited, mainly because of the low sensitivity of the technique.

In addition to tracking the transplanted stem cells after stroke, surveillance of the microenvironment and monitoring the therapeutic effect of the stem cells will play a major role in the assessment of clinical efficacy. Several preclinical studies have demonstrated the possibility of monitoring the inflammatory reaction after stroke by using USPIO particles. Ultrasmall SPIO–enhanced MR imaging allows noninvasive monitoring of macrophage recruitment into ischemic brain lesions. In particular, USPIO particles injected intravenously are phagocytosed by circulating macrophages, which subsequently can be visualized with T2*-weighted imaging once they are recruited to the injured brain. Findings in the preclinical experimental settings were reproduced in clinical studies.

Conclusions

Functional recovery after poststroke transplantation of NPCs cannot be therapeutically explained by cell replacement alone. As demonstrated by the transplantation of BMSCs, cell engraftment is not mandatory for functional recovery. Neural progenitor cell transplantation promotes CNS repair through additional alternative mechanisms, including the secretion of neuroprotective growth factors, induction of synaptic plasticity, angiogenic remodeling, and modulating deleterious components of the postischemic inflammatory response. In fact, the most efficacious treatments may require multiple transplantations of more and less differentiated NPCs combined with other somatic stem cell types and/or immune-based therapies. Intravascular administration might become a feasible route for stem cell delivery to the stroke-affected brain, with the advantage of minimal invasiveness. A great deal remains to be discovered. Ultimately, the development of clinically relevant strategies utilizing the full therapeutic gamut of human NPCs will require further understanding of what aspects of the host brain’s poststroke microenvironment are most conducive to cell-induced repair. Furthermore, the particular technique and timing of stem cell delivery should be used to maximize the therapeutic mechanism and advantageously utilize the reactive poststroke microenvironment.

References


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36. Hoehn BD, Palmer TD, Steinberg GK: Neurogenesis in rats after focal cerebral ischemia is enhanced by indomethacin. Stroke 36:2718–2724, 2005
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64. Park KI, Teng YD, Snyder EY: The injured brain interacts reciprocally with neural stem cells supported by scaffolds to reconstitute lost tissue. *Nat Biotechnol* **20**:1111–1117, 2002


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