

# New Approach in Ischemic Stroke Treatment: Stem Cell Therapy

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The aims of stroke management have evolved significantly during the past decade from localization and prevention to an attempt on acute treatment. A significant improvement on stroke outcomes at 3 months after the administration of intravenous tissue plasminogen activator was shown by two large randomized control trials.<sup>1,2</sup> The success of acute thrombolytic treatment has created an entirely new attitude towards stroke. In addition to strategies aimed at improving cerebral circulation, there has been increasing emphasis on neuroprotective therapies. The Stroke-Acute Ischemic NXY Treatment (SAINT I) Trial Investigators has demonstrated that the administration of NXY-059, a free-radical-trapping agent, within six hours after the onset of acute ischemic stroke significantly reduced disability at 90 days.<sup>3</sup> In spite of significant clinical benefits from acute treatment, many patients are still left with long-term neurological deficits due to onset-to-treatment time constraints. Once acute treatments have delayed and damages have maximized, little can be done to improve pre-morbid function.

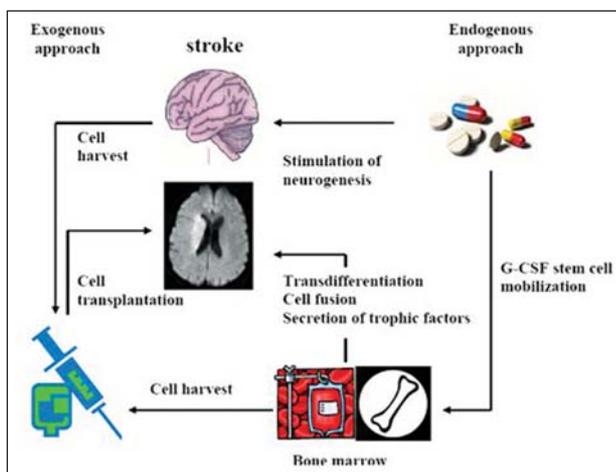
Recent attention has focused on restoring brain function through stem cell therapy. The main principle of utilizing cell-based therapies in ischemic cerebrovascular diseases is to replace infarcted neurons. Damaged nerve cells need to be replaced to allow the re-establishment of a functional neuronal circuitry. Several studies on animal model have shown that cells transplanted to the brain not only survive but also lead to functional improvement in different types of neurodegenerative disorders.<sup>4,5</sup> Clinical trials have supported the efficacy of intrastriatal fetal grafts in Parkinson's disease patients.<sup>6</sup> A few preclinical studies have shown the potential efficacy of neuronal transplantation in models of focal and global cerebral ischemia.<sup>7-9</sup> In this concise review, we focus on existing evidences that investigate various types of donor cells with the emphasis on autologous adult stem cells transplantation in both experimental and clinical models. Many factors affecting the graft survival, and efficacy of neural transplants including stroke subtype, location, severity and chronicity will also be discussed.

Neuronal stem cells are a subtype of neural progenitor cell in the nervous system that can self-renew and become both neurons and glia cells.<sup>10</sup> An ideal donor cell for intracerebral grafting would be able to proliferate, allowing ex vivo reproduction of high numbers of cells

from minimal donor materials. Potential stem cells to fulfill these criteria can be categorized based on their developmental origins into three different sources, namely: embryonic, fetal and adult stem cells. Pluripotent embryonic stem cells derive from blastocysts and have the capability to regenerate a primitive type of neural stem cells. Fetal stem cells are more restricted progenitor cells harvested from fetal organs undergo a major developmental process. Researches in transplantation of both embryonic and fetal stem cells are limited in a clinical setting since several controversies, i.e., ethical and legal issues, still remain. Thus, adult stem cells may be considered as an alternative option for cell therapy in stroke. The cells can be retrieved from bone marrow or certain areas of adult brain for example, subventricular zone and cingulate gyrus.<sup>11,12</sup> An approach to adult stem cells therapy can be categorized in to an endogenous and exogenous techniques. An endogenous approach is aimed to utilize adult stem cells that already physiologically exist in both the central nervous system and hematopoietic system. With the exogenous technique, the central nervous system or hematopoietic system derived stem cells are locally or systemically administered after the purification and multiplication process as shown in Fig 1.

The first human trial of cell therapy for stroke included 12 patients treated with neurons derived from a human embryonic carcinoma cell line, Ntera 2/cl.D1 (NT2).<sup>14</sup> This trial was not designed to examine efficacy; however, improvement in some patients on the European Stroke Scale scores and National Institute of Health Stroke Scale (NIHSS) was observed. As in Parkinson's disease, positron-emission tomography studies showed an increase in metabolic activity in the area of the grafts in several patients at 6 and 12 months after implantation. The results of an autopsy in one patient at 18 months after implantation documented survival of transplanted neuronal cells. Taken together, these data support the concept that the activity of implanted cells is responsible for clinical changes.

Bang and colleagues<sup>15</sup> performed a prospective randomized control trial aimed to test the feasibility, efficacy, and safety of cell replacement therapy using cultured autologous mesenchymal stem cells in patients with ischemic stroke. This study showed, long-term prognosis and neuroradiological features after intravenous injection of autologous mesenchymal stem cells in



**Fig 1.** An endogenous and an exogenous approach of autologous adult stem cell transplantation after stroke. (Adapted from Sebastian Haas, et al. *Curr Opin Neurol* 2005;18:59-64)<sup>13</sup>

patients with middle cerebral artery infarcts with severe neurological deficits. The study revealed an improvement in outcomes measured by Barthel index (BI) and modified Rankin scale in patients who received stem cell therapy when compared to control group (*p* value 0.011; 0.017; and 0.115 at 3, 6, and 12 months, modified Rankin score (*p* value 0.076; 0.171; and 0.286 at 3, 6, and 12 months, respectively). Serial evaluations showed no adverse cell-related, serological, or imaging-defined effects. Unfortunately, the number of patients were too small (*n*=5; and 25 in the treatment and control group, respectively) to draw any strong conclusion regarding the absolute benefits.

### From bench to bedside

In spite of the limitation of understanding of the basic knowledge of transplanted cells, stem cells transplantation in clinical trials has already begun. Many questions are still unanswered; for examples, what type of cell is most effective and safe? Which stroke patients should receive cell-based therapy? How the stroke location, severity and duration will affect the implanted cells? Will the inadequacy of blood supply that occurs after stroke influence the survival of the graft cells?

**TABLE 1.** Type of various graft sources under experimental and clinical trials

Graft source	Cell type	Model	Comments
Lateral ganglionic eminence <sup>7</sup>	Porcine, striatal progenitor	Animal	Graft differentiation into neurons and glia
Neural progenitor cell <sup>8,9</sup>	Rat, embryonic neural progenitor	Animal	Differentiate into all brain cell types in focal model
Bone marrow stromal cell <sup>6,17</sup>	Stromal cells from bone marrow	Animal	Functional benefit may be related to release trophic factors in focal model
Human umbilical cord blood cell <sup>18</sup>	Stem cells from umbilical cord blood	Animal	
Human bone marrow mesenchymal stem cells <sup>15</sup>	Autologous mesenchymal stem cell from bone marrow	Clinical Trial	Functional outcome (BI) was significantly improved in the interventional group.

### Cell types

Little is known concerning what cell type is the best to perform cell therapy in stroke. Several animal models have been tested as were shown in Table 1. However, one recent clinical trial has demonstrated that in patients with severe cerebral infarcts, intravenous infusion of autologous mesenchymal stem cells appears to be safe, feasible and may improve functional recovery.

### Duration of stroke: acute or chronic

The most appropriate time after stroke to initiate stem cell therapy is still unknown. Up to date, few investigations have aimed to answer the question of whether or not transplantation at different times after ischemic cerebrovascular event will affect proliferation, differentiation, integration and functional outcome. In one study using the porcine lateral ganglionic eminence as a cell source, equal graft survival and volume were obtained when the donor cells were transplanted at 3, 7, 14, or 28 days after stroke.<sup>7</sup>

Theoretically, transplantation to an acute infarct would be unlikely to succeed if there was a severe arterial blood flow disruption. Inadequacy of regional blood supply would not support graft viability. In addition, the release of free radicals, excitotoxic neurotransmitters and proinflammatory cytokines during an acute phase might contribute to an unsuccessful therapy. Ischemic changes may be a continuous process as Li and colleagues found evidences for apoptotic cells in the penumbra region persisting up to four weeks after stroke.<sup>19</sup> As for many of the above reasons, many investigators prefer to transplant at least months after the index event.

### Stroke location and site of implantation

The site of transplantation may determine the success of the therapy. Several studies have directly injected stem cells into the infarcted area where it remains unclear whether new tissue can survive. Hanadi et al. have demonstrated that fetal cortical grafts to ischemic rat brain survived in the ischemic penumbra but not in the infarcted core.<sup>20</sup> Therefore, it might be more appropriate to inject cells in the salvageable peri-infarct zone of the penumbra. However, transplanted cells might still be exposed to the deleterious effect of post-ischemic inflammation. An alternative approach is to deliver stem cells systemically using intravenous or intra-arterial route.

### Other considerations

The optimal number of transplanted cells needed to enhance the neurological recovery is still in question. This needs to be addressed taking into account the volume of the implanted cells might determine the route of the transplantation. Another important consideration is graft rejection and immunosuppressive medications if needed. Finally, the neoplastic potential of different graft sources especially multi-potential stem cells need to be better clarified. Embryonic stem cells have been demonstrated to form fetal teratomas at the transplantation sites in a Parkinson's animal model.<sup>21</sup> The formation of tumors would be a serious drawback to this mode of treatment.

## CONCLUSION

Stem cell transplantation is a novel treatment. Neural stem cells can be harvested from different sources and autologous stem cell transplantation might be feasible. Current evidences have shed some light on adult stem cell therapy in stroke patients. However, much work lies ahead to further clarify the biology of the different graft sources, the mechanism of action that promote structural and functional recovery and several clinical questions. Perhaps our greatest challenge is to establish a proportional significance of all mechanisms and to fine-tune our treatments to provide the greatest benefit for the victims of stroke.

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