Intravenous Cell Therapies for Stroke

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12.1 Introduction

The application of cell-based therapies is an emerging technology for cerebrovascular disorders, where there is unfortunately an urgent public health need for new treatments due to the limited endogenous regenerative capability within the brain (Williams and Hare 2011; Chen et al. 2003). For acute ischemic stroke, the predominant cerebrovascular disorder that is the leading cause of adult disability, tissue plasminogen activator is the only approved therapy which promotes recanalization of occluded cerebral arteries; however, only a minority of patients are eligible to receive it (Kleindorfer et al. 2008) because the drug must be administered within 3–4.5 h after symptom onset, according to regulatory guidelines. Once damage from stroke has maximized, little can be done to recover premorbid function. There are no approved effective treatments to reverse or repair brain damage associated with stroke. New therapeutic approaches using cells, rather than drugs, show much promise to promote repair of the injured brain. Among the various types of "cell therapies," there are different kinds of cells that fall into the categories of embryonic, fetal, and adult cell types, all of which are under development as potential new treatments for stroke. A growing body of extensive animal data suggest that cell therapies derived from a range of tissues (whether they are embryonic, fetal, or adult) improve neurological outcome in rodent models of stroke (Mattle and Savitz 2011; Savitz et al. 2011; Honma et al. 2006; Onda et al. 2007).

In this chapter, we discuss the intravenous delivery of cell therapies for stroke. There are currently multiple early phase studies in progress employing different routes of administration (intravenous, intra-arterial, intrathecal, and direct intracerebral transplantation). In stroke, there is an increasing emphasis on the intravenous use of cells for the following reasons: Intravenous (IV) administration is the least invasive and most practical among delivery routes and the most common and safest

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route for drug delivery. After stroke during the post-ischemic inflammatory response, there is upregulation of adhesion molecules, cytokines, and chemokines such as elevated SDF-1, which potently attract inflammatory cells and stem cells to the site of injury (Guzman et al. 2008). The chemotactic signals operating during inflammation and emanating from the brain can be leveraged to direct some types of intravenously injected cells to the damaged areas within the CNS. The intravenous delivery of various cell types has been reported to activate several different signaling mechanisms such as neuroprotective, immunomodulatory, and repair-enhancing processes in the brain. An intravenous administration could therefore lead to widespread cell distribution and consequent secretion of neuroprotective, proangiogenic, and immunomodulatory factors (Guzman et al. 2008). Lastly, there is an emerging literature that cells may exert potent effects on the immune response to stroke within peripheral tissues. Peripheral organs may be key therapeutic targets of systemically injected cells in animal stroke models.

12.2 Mechanism of Action of Stem Cell Therapy in Stroke

Multiple mechanisms of actions have been described after intravenous administration of cell therapies which we review here in more detail. These mechanisms include the following:

Neurogenesis and effects on astrocytes, *oligodendrocytes*, *and axons*: Some types of cell therapies stimulate the brain parenchyma to secrete neurotropic factors such as basic fibroblast growth factor and brain-derived neurotropic factor which activate pathways leading to enhanced survival, proliferation, differentiation, and migration of neural progenitor cells (Zhang and Chopp 2009). Some cell therapies decrease the astrocyte production of neurocan, which is an axon growth inhibiting proteoglycan, and some studies have reported an increase in axonal density around the ischemic lesions in the brain. An increase in the progenitor oligodendrocytes has been seen at the site of ischemic lesion after cell therapy which may enhance myelination. Hence, these mechanisms may play a role in the regeneration and repair process of cell therapy in ischemic stroke (Zhang and Chopp 2009).

Angiogenesis: Both angiogenesis and neurogenesis are closely interrelated and have been observed in the brains of patients with stroke. In a small study, there was a positive correlation between microvessel density and patient survival (Krupinski et al. 1994). There is increased synthesis of angiogenic growth factors such as FGF-2, PDGF, and VEGF and their receptors in the brain after stroke (Font et al. 2010). Injured brains after stroke in animals have shown an association with an increased level of these factors and increased angiogenesis (Zhang and Chopp 2009). Angiogenesis is directly linked to neurogenesis. The later needs new vasculature for prolonged survival. The mechanisms of angiogenesis are similar to neurogenesis and both processes share common factors. Both processes occur in the adult brain as a response to injury but can be stimulated by different types of cellular therapy (Font et al. 2010).

Immunologic mechanism: In rodent models, the adrenergic response post stroke has been associated with the release of immunological cells from the spleen which

contribute to secondary injury and exacerbation of the ischemic lesion. Intravenous umbilical cord blood cells prevent splenic release of immunological cells and decrease secondary injury in the brain (Walker et al. 2010). Different types of cell therapies have been shown to express anti-inflammatory cytokines which may even reduce brain damage due to post-stroke inflammation (Guzman et al. 2008).

12.3 The Perplexing Issue of Cell Trapping and CNS Entry

Despite extensive data attesting to brain remodeling that occurs after intravenous delivery of various types of cell therapies, the extent to which any intravenously administered cell type enters the brain has been a perplexing issue. Our studies and others have found that an intravenous administration of various different types of stem cells leads to their trapping in the lungs (Fischer et al. 2009). Cells lodge in the lungs temporarily and then migrate to other organs such as the spleen (Gao et al. 2001; Schrepfer et al. 2007). Cell size is a clear factor associated with lung trapping (Schrepfer et al. 2007; Harting et al. 2009) as many different types of purified and cultured stem cells have a cell size that is greater than the diameter of the pulmonary capillaries. Internal organs express high levels of SDF-1α, which can also direct cells to selectively home to their sites as well (Kucia et al. 2005). Adhesion molecules on the cell surfaces of capillaries may be another factor promoting lung trapping. Animal studies have shown that inactivation of the counter-ligand for VCAM-1 and CD49d significantly increases the passage of cells through the lung (Fischer et al. 2009). The redistribution of the cells after lung trapping is predominantly seen in the spleen and liver, to such an extent that it cannot be explained on the basis of cardiac output alone. Hence, it is presumed that pulmonary trapping of the cells might alter their ability for tissue homing, such that they migrate in increased numbers to the reticuloendothelial system (Fischer et al. 2009). We have identified that nitric oxide may be an important mediator that facilitates passage of bone marrow cells through the lungs by possibly stimulating vasodilation (Kasam et al. 2012).

Several investigators have begun to unravel the mystery how cells trapped within peripheral organs can still cause such profound effects within the brain. In an animal study of myocardial infarction, pulmonary passage of MSCs upregulated expression of multiple genes, with a large increase in the anti-inflammatory TGF-6 protein (Lee et al. 2009). There is also evidence from both human and animal studies that MSCs do not need to necessarily enter the injured area as they are capable of secreting paracrine factors which are responsible for anti-apoptotic actions leading to recovery (Mezey 2011).

MSCs are thought to exert multiple mechanisms in which they promote recovery. They may interact with immune cells in the reticuloendothelial system. They might induce or inhibit migration of different immune cells into the brain. We have found evidence in our early phase clinical studies that the spleen contracts in patients with acute ischemic stroke. At least in animals, the spleen releases immune cells into the circulation which migrate to the brain. This process might be affected by MSCs within the spleen and reticuloendothelial system. MSCs are thought to “sense” and

change their environment from a pro-inflammatory to anti-inflammatory milieu. They have been shown in animal models of GVHD to induce pro-inflammatory macrophages to become anti-inflammatory by secreting TGF-β and recruiting regulatory T cells (Mezey 2011). Preclinical studies have also shown that mesenchymal cells are effective in reducing lung injury from endotoxin, live bacteria, bleomycin, and hyperoxia (Matthay et al. 2010). Even neural stem cells when administered by IV routes have been shown to downregulate the inflammatory response emanating from the spleen in a model of intracerebral hemorrhage. Thus, multiple types of cell therapies may converge on the peripheral immune response (Lee et al. 2008).

12.4 Cell Types Under Investigation for Intravenous Delivery

Given more than a decade of research on cell therapies in rodent stroke models, a small number of clinical trials testing cell-based therapies in patients with ischemic stroke have been completed, and several more are underway or being planned. The most common cell types that have been brought forward to clinical trials using an intravenous route of delivery (Savitz et al. 2011) thus far are derived from the bone marrow and fall into two major categories. The first is the mononuclear fraction of bone marrow and the second is a more purified, cultured mesenchymal stem cell (MSC) population. Each has their own unique benefits and drawbacks. Mononuclear cells (MNCs) are composed of a mixture of myeloid, lymphoid, and stem cell populations (hematopoietic, mesenchymal, and endothelial); they can be rapidly prepared within hours of a harvest, do not require cell culture, and thus permit autologous administration, avoiding the potential for immunological rejection, a concern with the use of allogeneic cells (Savitz et al. 2011). MSCs, on the other hand, are more homogenous cell types derived from the mononuclear fraction, and their therapeutic applications in neurological disorders are highly supported by a large body of animal literature given their remodeling effects within the brain. MSCs need to be cultured and passaged for scaling to meet the requirements for a clinical trial. The culture conditions and number of passages can alter their biological properties. To date, it has not been possible to culture sufficient numbers of MSCs for an autologous application in the acute or subacute setting of stroke. Consequently, the first trials using autologous MSCs have been tested in patients with chronic stroke. Overall, we will first focus our discussion on early phase clinical studies involving stroke patients receiving intravenous cell therapies in which there have been four published clinical studies (Savitz et al. 2011; Lee et al. 2010; Honmou et al. 2011; Bang et al. 2005; Bhasin et al. 2011).

12.5 Clinical Trials: MSCs

Among the MSC clinical studies, all are from Asia and involve autologous applications. All were pilot trials and hence focused on safety and feasibility of administering intravenous stem cells to patients with ischemic stroke. Overall, there were no signals of safety concerns in any of these trials.

In South Korea, Bang and colleagues published an initial report on 5 patients given their own MSCs compared with 25 patients who were not given MSCs. The rationale for the number of patients in either group was not given. These patients had to have an ischemic stroke within 7 days prior to enrollment, and then they underwent a bone marrow harvest followed by MSC isolation and scale-up in culture. The MSCs were prepared in fetal bovine serum and took on average 30 days to grow to sufficient quantities for autologous infusion. Two doses of autologous MSCs were administered, the first at 4–5 weeks and the second dose at 7–9 weeks. The study patients were followed up to 1 year for safety evaluation. Subsequently, Lee et al. from the same group in 2010 describe the same patients with longer term follow-up and enrollment of more patients, totaling 16 study patients and 36 controls (Lee et al. 2010). There was blinded randomization to the two groups along with blinded outcome assessments. The control patients did not receive any additional interventions aside from standard of care. Twenty-one patients in the control group and 4 patients in the MSC group died but there was no statistically significant difference, although there was a trend toward decreased mortality in the stem cell group. There was no significant difference in the incidence of adverse reactions between the two groups. Of note, there were 5 patients who developed seizures in the control group and 3 in the MSC group. Similarly, there were 3 cases of recurrent vascular events in the controls including 2 with myocardial infarction and 1 with stroke, whereas there were 4 cases of recurrent vascular events in the MSC group out of which 2 were strokes and 2 were myocardial infarctions. Functional outcome on the mRS scale measured in the controls at a median time point of 3.5 years ranged from 2.7 to 4.9, whereas in the MSC group, it was measured at a median time of 3.2 years and ranged from 1.5 to 4.7. The number of patients in this trial was too small to draw any conclusions except that the study intervention appeared safe. Of note, no adverse effects related to fetal bovine serum were observed in any of the patients in the MSC group.

An observational study was then performed in Japan by Honmou and colleagues (2011) in which they enrolled 12 patients who had an mRS of 3 or greater, supratentorial strokes within the prior 6 months, no severe impairment of consciousness (as defined by Japan coma scale of between 0 and 100), and had an age between 20 and 75 years old. Patients who had extensive hemorrhagic transformation, infratentorial strokes, and any other organ dysfunction or severe medical comorbidities were excluded. The patients ranged from 41 to 73 years old with an average age of 59. The NIHSS varied from 2 to 20. Study patients were administered MSCs anywhere from 36 to 133 days after stroke. There was also variability in the dose of MSCs 0.6×10^8 to 1.6×10^8 cells per patient. A notable difference from other trials was that the cultured MSCs were grown in human serum, not fetal bovine serum. Cell passage was limited to three, and infusion occurred over 30 min. The main outcome assessed was safety as measured by neurological worsening, adverse reactions, and evidence of tumor or abnormal growth on MRI, none of which occurred. Clinical outcome assessed by unblinded physicians was measured by serial NIHSS and mRS just prior to cell infusion, immediately after cell infusion, and at several time points after infusion. At 1 year after infusion, the NIHSS ranged from 0 to 5 and the mRS ranged from 1 to 3. Eight out of the twelve patients achieved an mRS of 0–2 at

1 year. Radiological outcome was also measured by serial MRI scans which were interpreted by unblinded radiologists. Based on their assessments, mean lesion volume was reduced by >20 % at 1 week post infusion. This study adds further evidence for the safety of IV administration of MSCs in stroke patients.

Another small study on autologous MSCs was recently published by Bhasin et al. (2011) in India, comprising 12 patients with ischemic stroke within the prior 3–12 months. NIHSS score of enrolled patients was between 4 and 15. Patients were deemed eligible if they were able to comprehend. Patients were excluded if they had bleeding disorders, chronic liver and/or renal failure, progressive neurological worsening, unilateral neglect, neoplasia, contraindications to MRI, and immunosuppression. The selected 12 patients were divided up into 2 groups; half of them served as controls, while the other 6 received IV MSCs derived from their own bone marrow. The patients were followed at 8 and 24 weeks post infusion with laboratory, clinical, and radiological parameters to evaluate for safety. There were no differences clinically in the two groups.

12.6 Clinical Trials: MNCs

In contrast to MSCs, mononuclear cells represent a mixed cell population within the mononuclear fraction of bone marrow. Several randomized controlled clinical trials have reported that MNCs improve ejection fraction in patients with myocardial infarction. Various laboratories have published that MNCs when administered systemically improve neurological outcome in rodent stroke models.

Savitz et al. published a trial on the safety and feasibility of autologous bone marrow-derived MNCs in 10 patients with acute ischemic stroke. Patients were enrolled within 24–72 h of symptom onset, a time window which was felt to be the optimal window for efficacy based on animal studies. Patients underwent a bone marrow harvest (2 ml/kg draw) and then received an intravenous administration of purified autologous MNCs. As this was a safety and feasibility study only, there was no randomization and all patients enrolled received back their own cells. Outcomes were assessed at predetermined time periods at hospital discharge and then at 30, 90, and 180 days. The target maximum dose was ten million cells/kg. Eight out of ten patients received the target dose, but the other two received doses of seven million cells/kg and 8.5 million cells/kg, which represented the highest amount obtained from the harvest. Average age was 55 ± 15 years. There were no severe adverse events associated with the bone marrow harvest or infusion.

Collectively, these studies have begun to provide the first levels of evidence for safety of bone marrow-derived cells in patients with ischemic stroke. The MSC trials involved patients with chronic stroke, while the MNC trial involved patients with acute stroke.

There are a few other ongoing clinical trials of allogeneic MSC trials. Some of them involve chronic stroke and require the patient to have a stroke within the last 6 months, whereas others include patients with acute ischemic strokes. A list of trials can be found on clinicaltrials.gov.

Finally, the Athersys trial is a phase I/II dose escalation study, testing multipotential progenitor adult cells (MultiStem), another adherent stem cell population derived from the bone marrow of healthy volunteers. This study is ongoing and is in the early stages of development for stroke.

12.7 Major Issues That Need Further Study

12.7.1 Cell Type

What types of cell therapies are most suitable or appropriate for IV administration? At the present time, bone marrow and umbilical cord cells are the most conducive for IV. Various cells may exit the bone marrow and home to the brain after stroke. There is therefore an established endogenous mechanism already in place to support IV injections of bone marrow. Some have tested IV neural cells such as neural stem cells in rodent models of stroke (Zhang et al. 2004). Concerns do need to be addressed for the potential of NSCs to become trapped in the lungs and deposit in other peripheral organs.

12.7.2 Selection of Patients

What kind of patients should be included in cell therapy trials involving an intravenous delivery route? All trials thus far have focused on moderate to severe strokes defined either by the NIHSS or mRS. For example, the Japanese MSC study enrolled patients with NIHSS as low as 2, but the mRS was ≥ 3. All the trials restricted the upper age limit of the patients, at 75 in the Japanese and Korean studies, whereas Savitz et al. enrolled up to 80 years of age. As the aging population continues to grow, should we restrict the upper age cut offs? Patients with pulmonary and liver disease will likely need to be excluded at least in early stage safety testing as there is risk of exacerbating pulmonary diseases with entrapment of stem cells in pulmonary circulation.

12.7.3 Timing of Cell Therapy

Another major issue is when to enroll after stroke. The three studies varied greatly from acute stroke transfusion of MNCs by Savitz et al. (2011) to 1-year infusion of MSCs by (Lee 2010; Savitz et al. 2011; Bang et al. 2005), while the Japanese study by Honmou (2011) had a time interval somewhere in between (Honmou et al. 2011). The timing of cell administration should depend on the goal of treatment which spans several different areas from cytoprotection and immunomodulation to neuroregeneration/neurorepair. Some types of cell therapies may engage both sides of the spectrum. Animal studies would seem to suggest that if the former is the principal

goal, then the first few days to weeks is the optimal period. There is an evolving literature that some types of bone marrow preparations such as MNCs or umbilical cord cells when delivered intravenously have an outer limit of efficacy within the first few days (Yang et al. 2011; Iihoshi et al. 2004). Whether more purified cell types such as MSCs have longer windows when administered intravenously is an important issue. Some studies suggest that human MSCs can improve recovery even when given up to 30 days after stroke in rodents. This discussion raises the critical issue that the selection of a time window should be partly based upon animal data.

12.8 Efficacy in Clinical Trials

All clinical trials have been very small exploratory studies. Their main focus was safety and there was no definite study-related severe adverse reaction in any of them. Clinical outcomes were assessed differently in each of the studies, and again given the small number of patients involved and lack of controls, it is premature to offer any comments on efficacy at this time.

Conclusion

Intravenous delivery of cell therapies for stroke is a very practical method which is minimally invasive and supported by extensive animal data. To date, clinical trials have not identified clearly related severe adverse events after the intravenous administration of cell therapies in early phase clinical studies.

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