

Chapter

Transplanted Mesenchymal Stem Cells Aid the Injured Brain through Trophic Support Mechanisms

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ABSTRACT

Brain injury is a significant cause of death and permanent disability. Cell transplantation is a prospective treatment option because exogenous cells target a variety of pathological mechanisms in a sustained fashion and respond to injured brain tissue. Mesenchymal stem cells (MSCs) are an attractive cell source as they are relatively easy to obtain, expand and manipulate and pose minimal safety concerns. While MSCs may be able to transdifferentiate into neural cells, they are not likely replacing lost cells. Rather, MSCs secrete a plethora of soluble and insoluble factors that aid the injured brain by promoting cell survival and regeneration. This chapter reviews the role of transplanted MSCs in providing trophic support following brain injury.

INTRODUCTION

Brain injury occurs from either a traumatic (mechanical), ischemic (decreased oxygen; accounts for 83% of stroke cases), or hemorrhagic (ruptured blood vessel; accounts for 17% of stroke cases) insult to the brain. Stroke and traumatic brain injury (TBI) are major contributors worldwide to both deaths and persistent disabilities. Stroke is the third leading cause of death (behind heart disease and cancer) in the United States, with 137,000 Americans dying from stroke each year (Heron *et al.*, 2009). Stroke is the leading cause of serious, long-term disability in the United States. Currently, 795,000 people have a stroke each year and 15-30% of survivors have a permanent disability (Roger *et al.*, 2011). Annually, 1.7 million people sustain a TBI in the United States, resulting in 52,000 deaths and over 124,000 permanent disabilities each year (Faul *et al.*, 2010). Annual direct (e.g., medical) and indirect (e.g., loss of productivity) costs to the United States are \$41 billion and \$60 billion for stroke and TBI, respectively (Finkelstein *et al.*, 2006; Roger *et al.*, 2011).

The primary brain injury initiates a cascade of secondary events such as edema, excitotoxicity, and increases in free radicals, which act to spread the injury to surrounding tissue (for a review of the pathology, see Greve and Zink, 2009 for TBI and Mitsios *et al.*, 2006 for ischemic stroke). The brain attempts to repair and regenerate, but depending on such factors as injury severity, age of onset, and prior injuries, these endogenous attempts are often insufficient to restore normal function. A treatment that limits the spread of secondary damage and/or promotes repair and regeneration is needed. Current clinical treatment practices for TBI primarily aim to reduce intracranial pressure in an effort to minimize brain damage caused by swelling. For stroke, the only approved treatment is breaking down blood clots with tissue plasminogen activator. However, patients must meet strict criteria for receiving this therapy, including a 3 hour time window and no evidence of the following: bleeding, a severely elevated blood pressure or blood sugar, recent surgery, low platelet count, or end-stage liver or kidney disorders. Numerous pharmacological treatments that seemed promising in animal models have failed in clinical trials (Maas *et al.*, 2010; O'Collins *et al.*, 2006). Patients with brain injury vary widely with respect to demographics, severity of injury, location of injury, and co-morbidity factors making clinical trials challenging. Most treatments previously tested targeted pathways that are both deleterious and beneficial, making the dosage and time of treatment critical to not interfere with normal homeostasis or reparative mechanisms in the brain. Furthermore, these treatments targeted single mechanisms, which may not be enough in light of the multi-faceted pathology. Therapies that currently seem more promising, such as progesterone administration (Wright *et al.*, 2007) and cell transplantation, address multiple pathological events.

CELL THERAPY FOR BRAIN INJURY OVERVIEW

Cell transplantation offers the ability to target a variety of mechanisms in a sustained manner with a single therapeutic dose. Importantly, cells are living entities that may respond to the needs of the injured tissue via cell-cell signaling. Cell therapy has already shown promise for treating clinical stroke (Bang *et al.*, 2005; Kondziolka *et al.*, 2000) and TBI (Seledtsov *et al.*, 2005; Zhang *et al.*, 2008), and there are multiple ongoing clinical trials worldwide using various cell types to treat brain injury (www.clinicaltrials.gov).

MESENCHYMAL STEM CELLS FOR BRAIN INJURY

Mesenchymal stem cells are multipotent cells that can differentiate into cells of the mesoderm germ layer. These cells can be isolated from adipose tissue, amniotic fluid, placenta and umbilical cord, though are most commonly and efficiently derived from adult bone marrow. Marrow-derived cells that adhere to tissue-culture plastic *in vitro* are a heterogeneous population of cells that contain mesenchymal stem cells, but the entire population is more correctly defined as mesenchymal stromal cells (Horwitz *et al.*, 2005). As we learn more about these cell populations, the terminology evolves and the acronym MSC is used (and sometimes misused) for mesenchymal stem cell, mesenchymal stromal cell, multipotent stromal cell, and marrow stromal cell. For the purposes of this chapter, we will not distinguish amongst these cell populations and use MSC as a general acronym. MSCs are an attractive cell source because they are relatively easy to obtain, expand, and manipulate *in vitro*. In addition, adult MSCs do not have the tumorigenicity risks that pluripotent cells carry. MSCs home to sites of injury and cells delivered intravenously or intra-arterially are found to migrate to the injured brain tissue (Yagi *et al.*, 2010). There are advantages (e.g., minimally invasive) and disadvantages (e.g., pooling of cells in the lungs and spleen, requirement of high cell numbers) to delivery via the circulatory system and intracerebral delivery is another feasible option. While MSCs provide an autologous cell source, their immunoprivileged nature (Yagi *et al.*, 2010) makes them practical for allogeneic transplants as well. Using allogeneic cells allows for the ability to manipulate the cells without an extensive waiting period between cell harvest and transplantation and provides a cost-effective, off-the-shelf product. This feature is also attractive if multiple dosing is required for the therapeutic benefit to persist. Since ischemic and traumatic injuries are acquired disorders rather than progressively degenerative diseases, it is likely that a single dose will be sufficient. Ample preclinical data demonstrate that MSC transplantation promotes functional recovery following experimental cerebral ischemic or TBI (for review, see Parr *et al.*, 2007). Transplanted MSCs augment host repair and recovery primarily through direct and indirect cell-cell mediated trophic support. This chapter focuses on potential trophic support mechanisms provided by bone marrow-derived MSCs transplanted following brain injury.

TROPHIC SUPPORT MECHANISMS OVERVIEW

Trophic support classically means to provide nutrition, and can more broadly be characterized as promoting cellular growth, differentiation, and survival. MSCs secrete both soluble (cytokines,

growth factors) and insoluble (extracellular matrix proteins) factors that promote neural cell survival and regeneration (neuro-, angio-, and synaptogenesis) through paracrine signaling to neural and immune cells. Evidence of transplanted MSCs promoting survival and regeneration following experimental ischemic stroke or TBI is discussed below and potential factors that are mediating these effects are noted. A detailed list of trophic factors secreted by MSCs and their potential roles for aiding the injured brain is beyond the scope of this chapter, but more information can be found in Crigler *et al.*, 2006, Haynesworth *et al.*, 1996, and Tate *et al.*, 2010.

NEUROPROTECTION

Following the initial insult, secondary injury mechanisms persist and cause cell death to surrounding tissue. MSCs secrete multiple factors known to promote neural cell survival, including bone morphogenetic protein-4 (BMP-4), fibroblast growth factor-2 (FGF-2), fibronectin, glial cell line-derived neurotrophic factor (GDNF), heparin binding-epidermal growth factor-like growth factor (HB-EGF), hepatocyte growth factor (HGF), interleukin-8 (IL-8), nerve growth factor (NGF), and platelet derived growth factor (PDGF). There are several reports of decreased apoptotic markers and enhanced preservation of peripheral neurons when transplanting MSCs following experimental ischemic stroke (Li *et al.*, 2010; Xin *et al.*, 2010) or TBI (Kim *et al.*, 2010; Xiong *et al.*, 2009). Li *et al.* (2010) show that transplanting human MSCs into the injury penumbra 1 week following experimental cerebral ischemia in monkeys decreased apoptotic cell death and the lesion volume. Human MSCs transplanted into the injury cavity 1 week following experimental TBI in rats lead to enhanced cell survival in the hippocampus and improved functional recovery, and this was further improved when the MSCs were delivered within a collagen I scaffold (Xiong *et al.*, 2009). Kim *et al.* (2008) found that delivering human MSCs intravenously 1 day post-TBI in rats improved functional recovery and enhanced host cell survival by increasing pAkt and decreasing caspase-3 cleavage. Further, this group reports increases in brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and neurotrophin-3 (NT-3) in the brains treated with MSCs, though they did not distinguish whether these trophic factors were from the donor or host cells. Clearly, exogenous MSCs provide neuroprotection following brain injury and this is one mechanism of action for their benefit.

NEUROREGENERATION

After brain injury, the brain attempts to regenerate by resorting to a developmental-like state with increased neurogenesis, synaptogenesis, re-myelination, re-formation of the blood brain barrier, and angiogenesis. Once thought to be unable to regenerate, we now know that neural stem cells persist in the normal adult brain (neurogenic zones include the subventricular zone in the lateral ventricles and the subgranular zone in the dentate gyrus of the hippocampus). Neuroplasticity is the reorganization of neuronal circuitry by changing the number and strength of neurites and synapses. Such remapping occurs throughout life for learning and memory formation, and compensatory plasticity occurs in the spared tissue following brain injury (Nishibe *et al.*, 2010). After an ischemic or traumatic injury, endogenous neural stem cells proliferate, migrate to the site of injury, and differentiate into neurons and glia (Kernie and Parent, 2010).

Transplantation of MSCs augments endogenous regeneration following experimental ischemic stroke (Bao *et al.*, 2011; Li *et al.*, 2010; Xin *et al.*, 2010) and TBI (Mahmood *et al.*, 2004; Xiong *et al.*, 2009). Bao *et al.* (2010) show that intracerebral transplantation of human MSCs 3 days following experimental cerebral ischemia in rats increased proliferation and migration of host neural stem cells and also decreases their apoptosis, thus enhancing neurogenesis. They also report enhanced behavioral recovery and increases in brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and vascular endothelial growth factor (VEGF) in the brains of MSC-treated rats, though they do not identify the source of these cytokines. Xin *et al.* (2010) found that intravenous delivery of mouse MSCs 1 day following experimental stroke in mice lead to increases in axon fiber density, synaptogenesis and myelination. Following experimental TBI in rats, transplanted rat MSCs promoted increased proliferation and neuronal differentiation in neurogenic zones along with improved motor and sensory recovery (Mahmood *et al.*, 2004). Xiong *et al.* (2009) also report that transplanting human MSCs intracerebrally 1 week post-TBI in rats leads to increased axonal fiber length and that the fiber length was directly proportional to performance on the behavior tasks. Multiple trophic factors secreted by MSCs may contribute to enhancing neuroregeneration including brain derived growth factor (BDNF), fibroblast growth factor-2 , -7 (FGF-2, FGF-7), fibronectin , glial cell line-derived neurotrophic factor (GDNF), heparin binding-epidermal growth factor-like growth factor (HB-EGF), hepatocyte growth factor

(HGF), interleukin-6 (IL-6), leukemia inhibitory factor (LIF), monocyte chemoattractant protein-1 (MCP-1), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF).

Another important aspect of regeneration is angiogenesis (formation of new blood vessels). Transplanting MSCs increases angiogenesis following experimental ischemic stroke (Omori *et al.*, 2008; Onda *et al.*, 2008; Pavlichenko *et al.*, 2008) and TBI (Xiong *et al.*, 2009), and may involve hepatocyte growth factor (HGF), interleukin-6 (IL-6), matrix metalloproteinase-1 (MMP-1), and vascular endothelial growth factor (VEGF). Notably, many angiogenic cytokines also promote neurogenesis and neuritogenesis.

The glial scar that forms following brain injury acutely acts to sequester and clean debris at the injury site. Cellular components of the glial scar include reactive astrocytes, which help buffer excess glutamate and secrete neurotrophic factors, and activated microglia / macrophages which clear out dead tissue and secrete neurotrophic factors. However, extracellular components of the glial scar that persists adjacent to the injury site have been found to inhibit neurite extension (e.g., chondroitin sulphate proteoglycans, Nogo protein), thus limiting regeneration (for review, see Properzi *et al.*, 2003). Transplantation of MSCs helps overcome this glial scar limitation following experimental stroke (Li *et al.*, 2010; Li *et al.*, 2005; Pavlichenko *et al.*, 2008; Shen *et al.*, 2008). Following experimental ischemic stroke, rats treated with rat MSCs transplanted intravenously had decreased glial scar thickness at both the acute (3 and 6 days post-stroke; Pavlichenko *et al.*, 2008) and chronic (4 months post-stroke; Li *et al.*, 2005) phases. Along with decreased glial scar thickness, these studies report decreased lesion volume, enhanced regeneration, and functional recovery for animals treated with MSCs. Shen *et al.* (2008) show a decrease in neurocan (an inhibitory chondroitin sulphate proteoglycan) and enhanced axonal outgrowth in the injury penumbra when ischemic rats were treated with rat MSCs. Collectively, these data show that exogenous MSCs promote regeneration following brain injury.

IMMUNOMODULATION

There is a potent immune response following ischemic and traumatic brain injury. In addition to interacting with neural cells, MSCs communicate with immune cells and are now known to be immunosuppressive. Examining the interactions of MSCs with immune cells *in vitro* reveals that

MSCs suppress T cell proliferation and activation, inhibit B cell proliferation and IgG production, prevent dendritic cell differentiation and migration, and shift the cytokine secretion profile of dendritic cells, helper T cells, and natural killer cells towards anti-inflammation (reviewed in Mezey *et al.*, 2010 and Nauta and Fibbe, 2007). Interestingly, studies that separate the MSCs from the immune cells using semi-permeable membranes indicate that soluble factors are critical for these effects. Candidate factors include interleukin-6 (IL-6), interleukin-10 (IL-10), transforming growth factor β (TGF β), prostaglandin E2, hepatocyte growth factor (HGF), indoleamine 2,3-dioxygenase (IDO), and monocyte colony stimulating factor (M-CSF) (reviewed in Mezey *et al.*, 2010 and Nauta and Fibbe, 2007). Since shifting to a less inflammatory environment may promote neural repair and regeneration, immunomodulation is another potential therapeutic mechanism of action for transplanted MSCs.

CHALLENGES OF IDENTIFYING CRITICAL FACTORS

Cell transplantation is a dynamic treatment that can target multiple therapeutic mechanisms. Advantages of transplanting cells compared to pharmaceutical treatments include the ability to 1) easily localize the treatment to the affected tissue, 2) supply a variety of trophic factors at physiologic concentrations, 3) persist long enough to alter the microenvironment of the injured brain tissue; and 4) interact with host cells. The beneficial effects of transplanted MSCs have been repeatedly shown *in vitro* and *in vivo* and some potential pathways have been identified as described above. It is probable that a combination of multiple cytokines and mechanisms of action symbiotically contribute to improve functional recovery. While this ability to intervene along multiple pathways is desirable, it makes identifying key mechanisms and factors challenging. This becomes a hurdle for developing potency assays for the clinical use of MSCs. Potency assays are critical for ranking and qualifying different cell lots on their ability to promote recovery. Another complication for determining potency of cells *ex vivo* is that transplanted cells interact with the host cells via paracrine signaling and possibly direct cell-cell contact. MSCs alter the secretion profile of host neural cells (Xin *et al.*, 2010), which further acts to promote repair and regeneration. Additionally, the secretion profile of MSCs is a function of the microenvironment and changes in the presence of injured brain tissue (Chen *et al.*, 2002); and that should be modeled *in vitro*. Thus, there is a complex and dynamic web of factors and players involved in

cell-mediated effects. Elucidating critical aspects of this therapy will be the focus of intense research for years to come.

EXECUTIVE SUMMARY

- Stroke and TBI are major contributors to death and persistent disability.
- Cell transplantation is a promising treatment for brain injury and MSCs are an attractive cell source.
- MSCs secrete multiple soluble and insoluble factors that benefit the injured brain.
- *In vivo* data shows that transplanting MSCs enhances neuroprotection, promotes regeneration and/or suppresses inflammation.
- MSCs aid injured brain tissue by targeting multiple mechanisms, which is an advantage for a potential treatment, but a challenge for elucidating critical mechanisms and factors.

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