Global Journal of Animal Scientific Research

Journal homepage: www.gjasr.com

Print ISSN: 2345-4377 *Online ISSN: 2345-4385*

Mesenchymal Stem Cells in the treatment of Cerebral Ischemic Injury

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ARTICLE INFO

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How to cite this article:
Junior, Nilton B. A., Ricardo J. Del Carlo, Lukiya S. C. Favarato, Evandro S. Favarato, Vanessa G. Pereira1, Aline R. Murta, Betânia S. Monteiro and Daise N. Q. da Cunha. 2014. Mesenchymal Stem Cells in the treatment of Cerebral Ischemic Injury. *Global Journal of Animal Scientific Research. 2*(2): 83-91.

Article History:
Received: 9 April 2014
Received in revised form: 4 April 2014
Accepted: 15 April 2014

ABSTRACT

Mesenchymal stem cells (MSC) are undifferentiated adult stem cells capable of self-renewal and differentiation with a broad tissue distribution essential for tissue repairing and maintenance. These cells are isolated and expanded *in vitro* and kept as stem cells throughout many generations while maintaining its capability of differentiation when receiving appropriate stimuli. They have intrinsic multilineage potential, and as such, under special experimental conditions, are capable of differentiating into neuronal and glial cells, both *in vivo* and *in vitro*. The MSC migrate to the injured site after being intravenously injected, and in there promote endogenous cell proliferation, diminish apoptosis, and reduce the neurological deficitsresulting from cerebral ischemia. In this review we describe the many actions that the MSC exert on the injured nervous tissue, through their direct, paracrine, and systemic effects.

Keywords: Mesenchymal stem cells, cerebral ischemia, apoptosis, neuroprotection, neuroregeneration, angiogenesis, neurogenesis.

INTRODUCTION

The mesenchymal stem cells (MSC) have been found in a variety of tissues including adipose tissue, pericytes, muscles, organs and umbilical cord (Meirelles *et al*., 2008). In the bone marrow, they represent a rare population, less than 0.1% of nucleated cells. These cells have multilineage differentiating capabilities and participate reconstructing a variety of tissues (Pittenger, 1999; Argôlo Neto *et al*., 2012; Monteiro *et al*., 2012). These multipotent characteristics suggest that the MSC are responsible for repairing and maintaining all tissues in the body (Caplan, 2009).

The MSC play an important role protecting tissues, releasing growth factors, molecules and cytokines that allow local secretion of neurotrophic factors that enhances neurogenesis, and angiogenic factors that improve blood flow in the injured site through neoformation and reconstruction of the damaged vessels (Kinnaird *et al*., 2004 and Uccelli *et al*., 2011). Other roles of the MSC, through paracrine actions, include stimulation of synaptic connections and *remyelination* of damaged axons, reduction of apoptosis and regulation of inflammation (Seo e Cho, 2012). Even though these cells are not present in the ischemic site of injury, they are capable of secreting nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), and increase expression of anti-inflammatory cytokines such as IFN-γ, and IL-10, which may be beneficial for repairing and rearranging the neuronal connections; induction of regeneration; stimulating neurogenesis; axonal growth; and inflammatory response and tissue protection after spinal injury (Kurozumi *et al*., 2004; Lu *et al*., 2005 and Quertainmont *et al*., 2012). This suggests that the effects of cellular therapy in ischemia are not directly related to the presence of these cells in the brain, since there is a functional recovery even when there is no evidence that MSC are present in the cerebral parenchyma thus, indicating that they are capable of acting from a distance, i.e., by systemic immunomediated mechanisms (Borlongan *et al*., 2004; Bacigaluppi *et al*., 2009 and Brenneman *et al*., 2010).

In this review we discuss the main actions of the MSC associated with repairing and protecting the CNS from ischemic injuries (Figure 1).

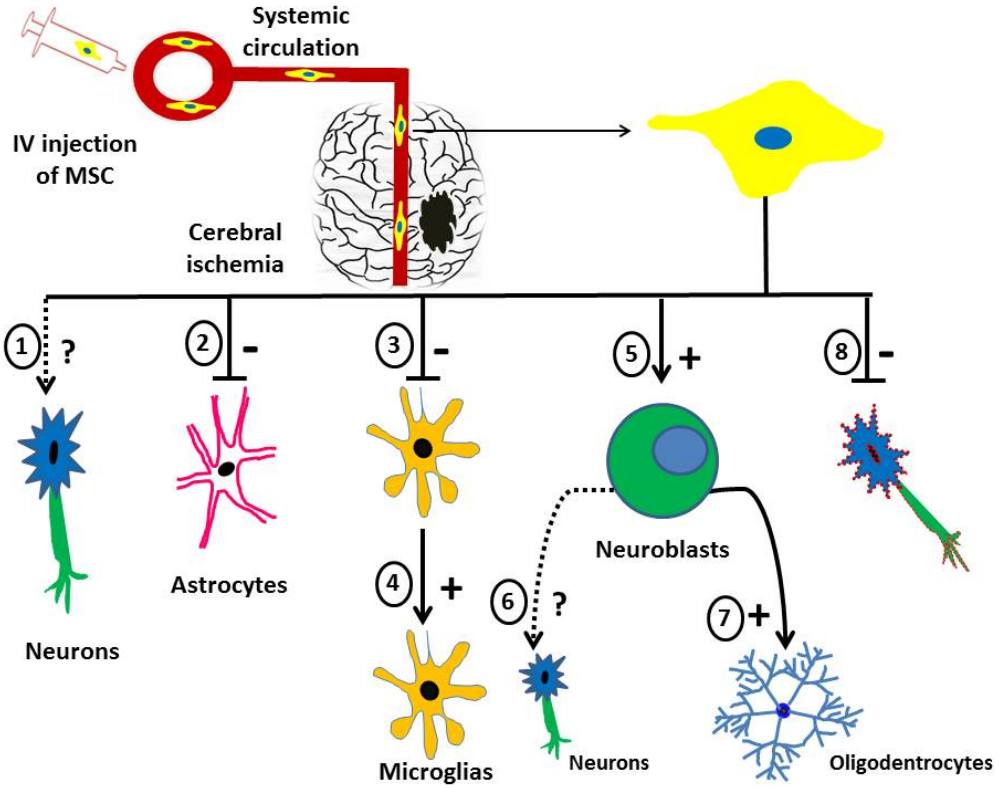

Figure1. The main neuroprotective effects of the MSC. 1- transdifferentiation, 2- astrocyte proliferation inhibition, 3- microglia proinflammatory response inhibition, 4- activation of microglial repair, 5- stimulates neuroblasts, 6- neuroblasts unknown action in neurons, 7- neuroblasts differentiation into oligodentrocytes, 8- inhibition of neuronal apoptosis. IV, intravenously, unknown mechanism; +, stimulation; - inhibition; dotted lines, indicate lack of strong evidence for the phenomenon to occur; solid line, known action. (Modified from Uccelli *et al*., 2011).

Modulation of the Inflammatory Response

Inflammation is one of the main consequences of cerebral ischemia due to the blood-brain-barrier rupture allowing for neutrophil and lymphocyte infiltration resulting in increased pro-inflammatory enzymes such as nitric oxide synthase (NOS) and proteases (Del Zoppo *et al*., 2000 and Wang *et al*., 2007). The MSC can be used for regeneration and repair therapy because they are capable to migrate to the injured site and have systemic immunomodulatory properties promoting effects on the tissue even when not present in the injured site (Bacigaluppi *et al*., 2009 and Quertainmont *et al*., 2012). They are capable to differentiate into neurons and glial cells, secrete cytokines, as well asneurotrophic and angiogenic factors that stimulate tissue repair and the migration of neuronal precursor cells (Li *et al*., 2002a and Wakabayashi *et al*., 2010).

Studies demonstrate that when MSC are transplanted into animals submitted to cerebral ischemia, these are capable to modulate the inflammatory process by reducing the buildup of Iba-1+, a microglia/macrophage-specific calcium-binding protein. Iba-1+ plays a role in the actin aggregation and participate in membrane ruffling, i.e., the formation of a motile cell surface that contains a meshwork of newly polymerized action filaments, which facilitate cellular migration and phagocytosis by the activated microglia (Ohsawa *et al*., 2004). Therefore, a reduction in Iba-1+, provided by the transplanted MSC, contribute to inhibit the pro-inflammatory expression that reaches not only the infarcted, but also the penumbra areacontributing to a reduction in ischemic expansion (SHEIKH *et al*., 2011). A significant reduction in the volume of the lesion is evident in the first few days and maybe associated with the greater production of neurotrophic factors by the MSC ensuring a neuroprotective action (Wakabayashi *et al*., 2010).

The MSC inhibit a series of pro-inflammatory molecules such as iNOS which reduces the production of NOS, cyclooxigenase-2 (Cox-2), IL-1β, IL-8, and monocytes chemoattractant protein-1 (MSP-1), which are capable of amplifying cerebral ischemia (Del Zoppo *et al*., 2000).

The activation of microglia may be modulated by neurons that inhibit inflammation (Tian *et al*., 2009). The neurons, as well as the endothelial cells diminish the microglial activation CD200dependent, a cell surface receptor that contains immunoglobulin domains, is found in the microglia and contribute to maintain themicroglia in a quiescent state (Amor *et al*., 2010). Similarly to neurons and endothelial cells, the MSC sufficiently increase the expression of CD200 in the presence of the, anti-inflammatory, cytokine IL-4, necessary to exert an anti-inflammatory effect modulating the microglia responses (Mcguckin *et al*., 2013).

Secretion of Neurotrophic Factors

The MSC favors themicroenvironmental conditions necessary to improve the region with cerebral damage by producing neurotrophic factors that protect or activate the endogenous reparation mechanisms of nervous tissue (Li *et al*., 2002). Substances that positively interfere in survival, differentiation and neuronal function of CNS are the neuronal growth factor (NGF), neutrophin-3 (NT-3), brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), hepatocyte growth factors (HGF), basic fibroblast growth factor (bFGF), and vasoendothelial growth factor (VEGF) (Uccelli *et al*., 2011). The NGF, secreted by the MSC, promotes phosphorylation, and thus, activates C reactive protein (PCR). The

phosphorylation of PCR stimulates the neuronal plasticity, acts in the regeneration capacity and in the prevention of the sympathetic neurons` death (Pierchala *et al*., 2004). Similarly, the BDNF plays a role on cell survival by promoting axonal regeneration, forming new synaptic connections (Coumans *et al*., 2001), and increasing the stimuli for the neural stem cells (NSC) differentiation, and also by protecting the neurons located in the damaged tissue (Barnabé Heider and Miller, 2003).

Comparative analysis demonstrate that bone marrow derived MSC release two folds more BDNF in relation to the adipose tissue derived MSC, and in general, cells derived from other types of tissues secret different growth factors. Therefore, the variations in neurotrophic factors from different MSC populations possess specific effects for each secreting cell types and may be chosen for a particular neurodegenerative disease (Razavi *et al*., 2013).

Studies in vivo demonstrate that MSC present Trk receptors, a family of tyrosine-kinase receptors that regulate the synaptic growth in the nervous tissue of mammals and participate in neuronal survival and differentiation. The Trk ligands are neurotrophins, a family of growth factors essential for CNS function. The NT-3, a neurotrophin that supports the neuronal survival, plays a role in the chemoattraction of the MSC due to its elevated affinity for Trk, contributing to MSC migration to the site of tissue damage (Chen *et al*., 2013).

The NSC release GDNG, which possesses high affinity for motor neurons, and warrant neuroprotection to monoaminergic and dopaminergic neurons in the nigro-striatal tract, and thus may be indicated for the treatment of degenerative diseases in this path, such as Parkinson´s disease (Whone *et al*., 2012). The GDNF´s expression significantly reduces apoptosis, providing neuroprotection to rats exposed to hypoxia (Yang *et al*., 2013), and yet increase the number of neuromuscular junctions (Suzuki *et al*., 2008).

The paracrine secretions of HGF by MSC significantly decreases demyelination due to a greater reactivity in the basic protein of myelin. The improvement of neurological recovery is attributed to the remyelination of nervous fibers and by axonal regeneration in vivo models of encephalic vascular ischemic and hemorrhagic accidents (Liu *et al*., 2010).In vitro data show that there are anti-apoptotic effects in neurons (Zhang *et al*., 2000a).

The bFGF is a polypeptide that promotes protection to the CNS cells. Its release by the MSC diminishes the infarct size in models of focal cerebral ischemia, such as in the rat. Also, it was demonstrated that intravenous administration of bFGF produces persistent reduction in the infarct volume at least up to three months after focal cerebrovascular accident (Sugimori *et al*., 2001).

The MSC transplant improves the angiogenesis after cerebral ischemia. However, it is impossible for the MSC to differentiate into endothelial cells forming new micro vessels due to the limited number of these cells. The MSC produce many growth factors, including VEGF that promotes angiogenesis in rat models of cerebral ischemia, and also significantly reduces functional deficits (Zhang *et al*., 2000b).Additionally, they play a role in the survival of nervous cells; stimulate axonal growth and the proliferation of the Schwann cells (Sondell *et al*., 1999).

Neurogenesis and Glial Activation

The subventricularzone (SVZ) in adult mammals contains neural stem cells (NSC) that differentiate and originate neuroblastsDcx+. In rats, the latter, in physiological conditions

migrate and reach the olfactory bulb. In models of encephalic ischemia, the neuroblats Dcx+, mediated by the factor derived from stromal cells 1α (SDF-1α) singnalingmigrate to the region suffering from ischemia. However, most of these neuroblasts die during their migration to the ischemic area due to apoptosis (Zhang *et al*., 2006). The MSC, implanted in models of ischemic injuries, influence the increase in NSC proliferation in the SVZ, and the survival of newly formed neuroblasts. One week after MSC infusion, there will be an increase in production of Dcx+ cells in the SVZ, thus intensifying neurogenesis. Therefore, the MSC increase the differentiation of the NSC due to secretion of growth factors favorable to neurogenesis, and promote the survival of neuroblasts that migrate to the ischemic area (Yoo *et al*., 2008).

Neurogenesis occurs in the SVZ and in the subgranularzone (SGZ) of the hippocampal dentate gyrus. However, the low survival rate of newly formed cells limits tissue repairing. In models of cerebral ischemia, the transplant of MSC increased proliferation and differentiation of NSC in the SVZ, increased the ratio of newly formed neurons, and of total cell proliferation. Histological analyses confirmthat transplanted cells had a significant survival rate at least three weeks after transplantation, and that it was possible to observe the expression of BDNF, which participates in neuronal migration (Kan *et al*., 2011). The NSC migrates in SVZ to the border zone of the ischemic area and differentiates into neurons, reduces apoptosis in rats treated with MSC improving the functional capacity after ischemia (Bao *et al*., 2011).

The astrocytes modulate the microenvironment around the neurons, ions flux, neurotransmitters, cell adhesion molecules, signaling molecules and release a great number of neuronal growth factors. In response to the ischemic event, in the second day, active astrocytes appear in the site of the lesion, and disappear five weeks later (Groves *et al*., 1993).Physiophathological studies demonstrate that only a few cells survive in the infarct area. After the third day, there is an increase in astrocytes that express glial fibrillary acidic protein (GFAP) and vimentin, mainly in the penumbra zone, and after the seventh day, these were found in the ischemic area as well (Li *et al*., 2005 and Wakabayashi *et al*., 2010). Reactive astrocytes are characterized by an intense immunoreactivity to the GFAP protein and are responsible for forming the glial scar. However, the intense presence of astrocytes in the penumbra zone inhibits the growth and regeneration of axons (Fitch E Silver, 2008).

The MSC implant, in models occluding the medial cerebral artery, demonstrated that after the third day there was a buildupreduction of GFAP+ astrocytes in the penumbra zone, and in the seventh day in the ischemic area (Sheikh *et al*., 2011). The decreasedthickness of gliosis allows axonal growth and formation of new synapses (Li *et al*., 2005).

Studies demonstrate increased expression of GAP-43 in the axons of neurons in the SVZ of rats. The Gap-43 is an essential protein for the axon and pre-synaptic region where high levels are expressed in neuronal growth cones during development and axonal regeneration (Benowitz and Routtenberg, 1997).The MSC propitiate the development of new axonal connections with intracortical (in the penumbra zone) axonal projections constituting a new network among the neurons promoting functional neurological recovery (Li *et al*., 2005).When infused after cerebral ischemia in rats, GAP-43 induces differentiation of the NSC in oligodendrocytes affording an improved functional recovery, possibly due to oligodentrogenesis stimulation (Rivera *et al*., 2006).

Angiogenesis Induction

The angiogenesis, that occurs in cerebral ischemia aid blood flow restoration improving the offer of oxygen and nutrients to the affected tissue, and thus is essential for neurological recovery (Krupinski *et al*., 1994). In patients with encephalic vascular accident (EVA), the degree of angiogenesis is correlated with survival, mainly in those with a greater microvessel density in the penumbra zone (WEI *et al*., 2012). In animal models, the angiogenesis may be amplified with MSC treatment because these cells migrate to the injured site of the nervous tissue (Li *et al*., 2002b), andrelease growth factors such as VEGF and bFGF (Chen *et al*., 2003).

Rats with induced EVA and treated with MSC had an increase in the number of VEGF-positive cells distributed throughout the ischemic cortex, detected by quantitative and immunofluorescence analysis (Guo *et al*., 2012).

After cerebral ischemia, the MSC proliferate and remodel the cortex microvasculature improving collateral blood flow, which allows identifying the presence of angiogenic factors in the penumbra zone (Whitaker *et al*., 2007 and Wei *et al*., 2012). It was demonstrated that after the transplant, in hypoxic condition, VEGF synthesis is elevated and bone marrow derived MSC are stimulated to differentiate into endothelial cells favoring angiogenesis and the performance during neurological and behavioral tests (Li *et al*., 2002b and Caplan and Dennis, 2006).

Antiapoptotic Effect

The death of neurons and glial cells are reduced by the trophic factors secreted by MSC which throughparacrine effectsincrease the survival of nervous cells in the cerebral ischemic site and reduces apoptosis (Chen *et al*., 2003 and Caplan e Dennis, 2006). The protection of the cortical neurons by the MSC in models of ischemia could be mediated by different mechanisms, such as direct MSC effects on the neurons and by secretion of factors that stimulate astrocytes to produce neuroprotective factors (Scheibe *et al*., 2012).

One of the main mechanisms that underlie the antiapoptotic effects of the MSC is the increase in NGF, BDNF, and NT-3 that activate an Akt-dependent pathway, also known as kinase protein B. The Akt protein modulates a large number of molecules that participate in cell proliferation and also inhibits apoptosis (Inoki *et al*., 2002). The MSC significantly super express Akt gene after the second day of the ischemic injury returning to basal levels in the eighth day. During this period apoptosis is inhibited (Kim *et al*., 2010).

CONCLUSION

The MSC participate in tissue protection, releasing growth factors, molecules and cytokines that allow, in the site of tissue damage, secretion of neurotrophic factors that favor neurogenesis, and angiongenic factors that improve blood flow due to neoformation and/or reconstruction of damaged vessels. Besides neuro and angiogenesis, the MSC also potentiate the formation of synaptic connections and remyelination of injured axons, reduce apoptosis and diminish inflammation. Furthermore, these cells are capable of acting from a distance modulating the action of the immune system.

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