

Review

Mesenchymal Stem Cell Therapy for Ischemic Stroke

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To date, many animal studies have indicated the neuroprotective effects of mesenchymal stem cell (MSC) transplantation in ischemic stroke. Several clinical studies have also revealed the safety, feasibility, and neuroprotective effects in ischemic stroke patients. In this review, we present the main approaches of MSC transplantation in ischemic stroke, the mechanisms of MSC therapy, and the current clinical studies on MSC transplantation in ischemic stroke patients. We also explore the safety of MSC transplantation and conclude that MSC therapy will play an important role in the future treatment of ischemic stroke. The optimal timing, approach, and cell dose in the transplantation are important issues for successful clinical application.

Key words: mesenchymal stem cell, ischemic stroke, cell transplantation, clinical trial

Ischemic stroke is a major cause of morbidity and mortality, and there are limited therapeutic options. Because of the short therapeutic time window in ischemic stroke, many patients suffer from severe disabilities, even if they receive timely interventional or thrombolytic therapies [1]. In recent years, stem cell transplantation has emerged as a new approach for the treatment of ischemic stroke [2-6]. There are several cell sources for stem cell therapy for ischemic stroke. Among them, mesenchymal stem cells (MSCs) have the advantage of being rapidly isolated from bone marrow and do not require a long culture time, making them well suited for autologous administration even in the acute phase of stroke [7]. MSC transplantation approaches include intracerebral, intravenous, and intra-arterial transplantation [4,5,8-26]. The mechanisms of MSC transplantation for ischemic stroke are mainly considered based on their neuroprotective effects [2-4,8,10,14,16,17,21,22,25,27-30]. MSCs

can differentiate into various types of cells, such as endothelial cells and neuronal cells [3,28]. Moreover, the benefits of MSC transplantation have been ascribed to the secretion of neurotrophic factors or chemotactic cytokines from transplanted MSCs [2,3,24,28,30]. In addition to the secretory function, migration of MSCs into the infarct area and the reduction of the infarct volumes after transplantation can mediate the neuroprotective effects [2-4,14,28]. Therefore, both animal and clinical studies have confirmed the therapeutic effect of MSC transplantation in ischemic stroke [1]. In the past few years, some preliminary phase I/II trials have identified the safety and feasibility of autologous MSC transplantation in ischemic stroke patients [5,31-34,48,51,55,56]. In this review, recent findings on MSC therapy are described, as well as the current status of MSC therapy for ischemic stroke.

MSC Transplantation Approaches

Intracerebral transplantation. The advantage of

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intracerebral transplantation is the targeted deposition of stem cells into the lesioned brain, compared to intravascular transplantation (intravenous or intra-arterial transplantation), which results in the majority of the cells being lodged in peripheral organs [12,26]. However, intracerebral transplantation is more invasive than intravascular transplantation, with the added possibility of adding new injury to brain tissue [35].

Intravenous transplantation. Intravenous transplantation is the simplest and safest method, compared to intracerebral or intra-arterial transplantation [11]. Therefore, intravenous MSC transplantation was most commonly used for ischemic stroke animals and patients in the past. Unfortunately, the transplanted cells must pass through the systemic and pulmonary circulation systems. Cell replacement is therefore difficult to achieve by intravenous MSC transplantation, and there are trophic effects of MSCs to consider as well [2, 11].

Intra-arterial transplantation. Intra-arterial transplantation falls somewhere between intravenous and intracerebral transplantation due to it being relatively less invasive and the number of cells reaching the lesion site. A higher number of human MSCs migrated into the infarct lesion with intra-arterial transplantation than with intravenous transplantation, resulting in better functional recovery in animal stroke models [4, 14]. On the other hand, intra-arterial transplantation carries the risks of cerebral embolism and the reduction of cerebral blood flow associated with microstrokes [36].

Therapeutic Effects of MSC Therapy on Ischemic Stroke Model of Animals

Mechanisms of MSC therapy. MSCs contribute to the treatment of cerebral ischemia through multiple mechanisms, including cell migration, angiogenesis, prevention of apoptosis, secretion of neurotrophic factors, neural circuit reconstruction, and immunomodulation [1]. Mainly, MSCs exert neuroprotective effects through cell migration into the infarct area with the subsequent secretion of neurotrophic factors.

MSC migration into the infarct area. Previous reports have revealed that transplanted MSCs mainly migrate into the ischemic penumbra and the subventricular zone [1, 37, 38]. Microglia and astrocytes in the infarct area secrete stromal cell-derived factor 1 (SDF-1). MSCs express chemokine receptor 4 (CXCR-4), the physiological receptor for SDF-1. The interaction of SDF-1 and CXCR-4 causes MSC migration into the

infarct area [1, 39, 40]. A lack of CXCR-4 or SDF-1 α will significantly reduce the targeted migration of MSCs [41]. These mechanisms are one of the causes of MSC migration. However, the manner in which MSCs pass the blood-brain barrier remains poorly understood.

Neurotrophic factors secreted by MSC. Several neurotrophic factors have been shown to contribute to the therapeutic outcomes of stem cell therapy in experimental stroke. *In vitro* studies show that MSCs secrete at least 11 kinds of neurotrophic factors after co-culture with cortical neurons under hypoxic conditions [42]. MSCs have neuroprotective effects in the early stage of transplantation in rats with cerebral ischemia [43]. MSCs also induce parenchymal cells in the host tissue to secrete nerve growth factor, brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), epidermal growth factor, basic fibroblast growth factor, insulin-like growth factor 1, hepatocyte growth factor, and stem cell factor [4, 28, 44, 45, 46, 47]. These neurotrophic factors promote functional recovery after stroke through the reduction of apoptosis and inflammation, proliferation of endogenous stem and progenitor cells within the peri-infarcted tissue, angiogenesis, and neurogenesis [1, 28-30, 35].

Clinical application of MSC transplantation for ischemic stroke patients. In Table 1, recent clinical trials involving MSC transplantation in ischemic stroke patients are listed. Initially, there were a few reports on intracerebral transplantation. In one study, five chronic stage patients received stereotactic transplantation of autologous bone marrow stem cells [48]. There were no adverse events after surgery, and some patients showed improved neurological function. However, because of the small number of patients, these results demonstrated only the safety of intracerebral transplantation. In contrast, there are several studies on intravenous transplantation as a less invasive method. In 2005, autologous intravenous MSC transplantation was started for ischemic stroke patients. In this phase I randomized study, five patients received intravenous administration of 1×10^8 MSCs with subsequent functional recovery [49]. There were no adverse events, such as venous thromboembolism, abnormal cell proliferation, systemic cancer, systemic infection, or neurological decline after intravenous MSCs transplantation [50, 51, 52]. In 2014, the first phase II randomized clinical trial of intravenous allogeneic MSC transplantation in

Table 1 Recent clinical trials with MSC transplantation in ischemic stroke patients

Author	Number of patients	Cell type	Route	Cell dose	Time window	Result
Bang 2005 [49]	5	MSC	IV	1×10^8	N/A	Safe and effective
Suárez-Monteagudo 2009 [48]	5	BMSC	IC	$1.4\text{--}5.5 \times 10^7$	1–10 years	Safe
Bhasin 2011 [51]	6	BMSC	IV	$5\text{--}6 \times 10^7$	6 months–1 year	Safe and feasible
Savitz 2011 [34]	10	BMNC	IV	10 million cells/kg	24–72 hours	Safe and feasible
Battistella 2011 [55]	6	BMNC	IA	1.5×10^8	Within 90 days	Safe and feasible
Moniche 2012 [5]	10	BMNC	IA	1.59×10^8	5–9 days	Safe and feasible
Friedrich 2012 [56]	20	BMNC	IA	2.3×10^8	3–7 days	Safe and feasible
Diez-Tejedor 2014 [53]	10	MSC	IV	N/A	2 weeks	Safe and effective
Moniche 2015 [57]	76	BMNC	IA	2×10^6 /kg or 5×10^6 /kg	N/A	Ongoing
Steinburg 2016 [58]	18	modified MSC	IC	2.5×10^6 or 5.0×10^6 or 10×10^6	2 years	Effective
Moniche 2016 [7]	22	BMNC	IA	1.53×10^8	5–9 days	Effective
Honmou. 2016 [54]	N/A	MSC	IV	N/A	Within 40 days	Ongoing

BMNC, bone marrow mononuclear cell; BMSC, bone marrow stem cell; MSC, mesenchymal stem cell; IA, intra-arterial; IC, intracerebral; IV, intravenous.

ischemic stroke patients was reported [53]. In this study, the therapeutic effect of intravenous allogeneic MSC transplantation was shown in sub-acute stage patients. These results indicate the safety, feasibility, and therapeutic effects of intravenous MSC transplantation in ischemic stroke patients. For over a decade, MSC transplantation has been shown to have therapeutic effects on stroke patients. Moreover, a phase III randomized clinical trial of intravenous allogeneic MSC transplantation on chronic stage patients is ongoing [54]. On the other hand, several clinical studies have suggested the beneficial neuroprotective effects of intra-arterial MSCs transplantation for ischemic stroke patients in recent years. The initial studies have shown that autologous intra-arterial MSC transplantation in ischemic stroke patients in the sub-acute phase was safe and feasible and free of adverse events [5, 55, 56]. In one study, patients with moderate to severe ischemic stroke were treated within 3 to 7 days after onset. No

observable complications were found, and 40% of the patients showed a good clinical outcome at the chronic phase [53]. However, the optimal time window is still unclear. It is very difficult to argue the timing of cell transplantation for stroke patients because the condition of patients with ischemic stroke varies remarkably. A clinical trial of intra-arterial MSC transplantation in acute ischemic stroke is ongoing [57]. Furthermore, the current study revealed a strong correlation between intra-arterial transplanted cell dose and patient outcome [7]. A cutoff point of 310×10^6 injected cells predicted a good outcome with 80% sensitivity and 88.2% specificity, although additional basic and clinical studies are needed. A very recent phase I/II study of intracerebral cell transplantation for stroke patients reported positive data using genetically modified MSCs [58]. This study showed the safety and clinical improvement at 12 months after transplantation. The neuromodulation by the cell transplantation might be strongly related

to the therapeutic effects. We are now proceeding to an international multi-center clinical trial for traumatic brain injuries using these promising genetically modified MSCs.

Conclusions

The main approaches of MSC transplantation for ischemic stroke, the mechanisms of MSC therapy, and the current clinical studies have been shown in this review article. To date, many animal studies and several clinical studies have investigated the optimal timing, approach of transplantation, and cell dose. However, all of these characteristics are still undetermined. Currently, a phase III, randomized clinical trial focusing on intravenous transplantation and a phase II, randomized clinical trial focusing on intra-arterial transplantation are ongoing. Additional basic and clinical studies are needed for future clinical application.

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