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·Review·

Bone marrow stromal cells as a therapeutic treatment for ischemic stroke

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Cerebral ischemia remains the most frequent cause of death and quality-of-life impairments due to neurological deficits, and accounts for the majority of total healthcare costs. However, treatments for cerebral ischemia are limited. Over the last decade, bone marrow stromal cell (BMSC) therapy has emerged as a particularly appealing option, as it is possible to help patients even when initiated days or even weeks after the ischemic insult. BMSCs are a class of multipotent, self-renewing cells that give rise to differentiated progeny when implanted into appropriate tissues. Therapeutic effects of BMSC treatment for ischemic stroke, including sensory and motor recovery, have been reported in pre-clinical studies and clinical trials. In this article, we review the recent progress in BMSC-based therapy for ischemic stroke, focusing on the route of delivery and pre-processing of BMSCs. Selecting an optimal delivery route is of particular importance. The ideal approach, as well as the least risky, for translational applications still requires further identification. Appropriate pre-processing of BMSCs or combination therapy has the benefit of achieving the maximum possible restoration. Further pre-clinical studies are required to determine the time-window for transplantation and the appropriate dosage of cells.

Keywords: bone marrow stromal cell; cerebral ischemia; transplantation; neuroprotection; administration method

Introduction

Neuronal necrosis results in permanent neurological deficits that frequently lead to morbidity or mortality following cerebral ischemia. Despite timely treatment with thrombolysis and percutaneous intravascular interventions, many patients are often left with irreversible neurological deficits. Current therapy for ischemic stroke is limited to acute measures designed to restore perfusion, promote circulation, and protect ischemic cells from death in the acute phase. In contrast, little attention has been paid to enhancing long-term repair and recovery of the brain after ischemic stroke. Neuroregeneration has recently attracted much attention because of the regenerative capacity of neural tissue[1]. Treatments are focused on the effective control of risk factors associated with cerebrovascular disease, preventive pharmacotherapy, and lifestyle modification. Now, stem-cell therapy is being used in the treatment of ischemic stroke.

Stem cells, due to their multipotency, have been used to either replace lost neurons or to stimulate the regeneration of cells to treat cerebral ischemia[1]. A key to the efficacy of bone-marrow stromal cells (BMSCs), also referred to as mesenchymal stromal cells (MSCs), is that they have the potential of self-renewal and multilineage differentiation, thus rendering them capable of differentiating into neuronal and glial lineages under specific conditions[2]. In cerebral ischemia, BMSCs secrete many neurotrophic factors, develop neuronal and vascular markers, and aid in repair of the ischemic brain. Recently,

several experimental studies have demonstrated that BMSCs grafted into the ischemic brain of rats ameliorate somatosensory and motor impairments, and improve the modified neurological severity score (mNSS), and BMSC delivery has been reported not only as an effective reparative treatment, but also as a protective therapy[1-4]. Many clinical trials have recently demonstrated that BMSC therapy appears to be safe and feasible for the treatment of ischemic stroke[75-78]. There are currently three major routes for BMSC transplantation: intracranial (i.c.), intra-arterial (i.a.), and intravenous (i.v.). In this review, we comprehensively evaluate the safety and efficacy of the different routes of transplantation, the methods of pre-processing of BMSCs in contrast to unprocessed BMSCs, and combination therapy involving BMSCs.

Biological Characteristics of BMSCs

There are two major cell types in human bone marrow, hematopoietic and non-hematopoietic stem cells. BMSCs, a type of multipotent stem cell[5], have the ability to adopt the fate of mesodermal, endodermal, and ectodermal cell types[6]. They are also able to differentiate into non-mesenchymal lineages including neurons and glial cells, as well as mesenchymal cell lineages including cardiomyocytes[7], osteoblasts, chondrocytes, adipocytes[3], fibroblasts and hepatocytes in specific microenvironments[8]. The ability for multilineage differentiation of BMSCs renders them potentially useful in treating various diseases[9]. Indeed, experimental studies have reported that BMSCs can ameliorate neurologic deficits and facilitate functional recovery in many disorders of the central nervous system, such as Parkinson's disease[10], trauma, spinal cord injury, multiple sclerosis, and cerebral ischemia[9].

Pre-clinical Studies of BMSC Transplantation for Ischemic Stroke

BMSC transplantation has a potentially important therapeutic effect on cerebral ischemia in animal models[11]. Grafted BMSCs secrete nerve growth factors and/or neurotrophic factors, reduce the infarct volume, promote angiogenesis[12], and improve functional recovery in rat models after ischemic stroke. In a rat model of transient ischemia established by middle cerebral artery occlusion (MCAO)[13], administration of BMSCs ameliorates the behavioral impairments and reduces infarct volume, and these effects are associated with the promotion of endogenous neuronal stem cell differentiation, and the survival of newborn neuroblasts. While the mechanisms underlying the functional recovery remain unclear, BMSC therapy may provide a new avenue for the treatment of cerebral ischemia.

Optimum Delivery Routes and Time Window of BMSC Transplantation

BMSC transplantation at different time points improves functional outcomes in animal models of stroke using three transplantation routes: i.c., i.a. and i.v. (Table 1). The i.c. route involves the direct injection of BMSCs (at a specific volume and quantity) into brain parenchyma or a lateral ventricle with the assistance of stereotactic surgery, ensuring that a high concentration of the grafted cells is directed to the target point. This route can be mainly divided into intrastriatal and intracerebroventricular (i.c.v.) approaches. In the study by Li *et al.,* bromodeoxyuridine (BrdU)-labeled BMSCs transplanted directly into the striatum of adult mice after MCAO showed significantly increased axonal sprouting and remyelination in the cortical penumbra[14], along with improved rotarod test performance and mNSS compared with the controls. Intrastriatal injection has advantages over i.c.v. administration, in that it takes less time to produce effects[15, 16] and requires a smaller number of cells (usually $1–2\times10^5$)[14, 15]. Another study found that intrastriatal injection of BMSCs decreases matrix metalloproteinase (MMP) activation and improves neurovascular unit disruption after transient (2 h) MCAO[17]. However, cells administered through the i.c.v. route adhere to the ventricular wall, penetrate the ventricular surface to the lesion, and reduce behavioral deficits[18]. Therefore, this method appears to be less invasive, produces more extensive cell seeding, and permits the administration of more cells than intrastriatal injection. In a study by Zhao *et al*., at 2 weeks after implantation of purified human BMSCs (hMSCs) into rat cortex surrounding the ischemic zone, the rats showed improved performance in the limb placement test[19]. Six weeks later, they demonstrated improved functional performance.

The optimal timing and cell-dose of BMSCs administered *via* i.c. for ischemic stroke remain unclear.

Table 1. Optimum delivery routes and time-window of BMSC transplantation

Stroke model	Delivery route	Timing	Results	Ref
Permanent MCAO	i.c. (striatum)	3/4/7 days	BMSCs survived, migrated towards ischemic boundary zone (IBZ), expressed neuronal or astrocytic markers, increased axonal sprouting, and remyelination occurred in the cortical penumbra	[14, 20, 21]
Transient MCAO (2 h)	i.c. (IBZ)	24 h/3 days	Enhanced endogenous neurogenesis; increased MMP9 activation, stimulating neurons to modulate cerebral blood flow	[13, 22]
Transient MCAO (2 h)/ Permanent MCAO	i.c. (striatum)	24 h/7 days	Inhibited pathologic upregulation of brain-isoform glucose transporters types 1 and 3, and promoted recovery of local glucose metabolism in peri-infarct area	[9, 23]
Permanent MCAO	i.c. (lateral ventricle)	24 h	BMSCs survived, adhered to ventricular wall, and clearly reduced behavioral functional deficits	[18]
Cortical ischemia	i.c.	7 days	hMSCs survived, expressed markers of neuroectodermal cells, and ameliorated neurological deficits	[19]
Transient MCAO (2 h)	i.a.	24 h	BMSCs distributed throughout the area of MCA, up-regulated the expression of BMP 2 and 4, gap junction protein connexin-43, and synaptophysin	[5, 24]
Transient MCAO (2 h)	i.a.	24 h/2 h	Reduced lesion size, increased axon-myelin remodeling which may contribute to functional restoration	[16, 25]
Transient MCAO (2 h)	i.v.	24 h	Produced many trophic factors; monocyte chemoattractant protein-1 levels significantly increased from 6 h, peaked at 48 h after MCAO, and enhanced BMSC migration and tissue repair	[26, 27]
Transient MCAO (2 h)/ Permanent MCAO	i.v.	24 h	hMSCs expressed bFGF and VEGF, promoted expression of insulin-like growth factor 1 and receptor, which contributed to improved recovery and increased neurogenesis	[28-30]
Transient MCAO (2 h)	i.v.	24 h	Cell-free MSC-generated exosomes survived, enhanced neurite remodeling and angiogenesis, and improved functional recovery; male MSCs increased bFGF expression, reduced apoptosis, and improved functional recovery in female rats	[31, 32]
Transient MCAO (90-min)	i.v.	3 h	Down-regulated expression of survivin and Bcl-2, prevented apoptosis and cell death	[33]
Global cerebral ischemia	i.v.	3 h	Reduced infarct volume and neuronal loss, increased BDNF expression, ameliorated functional deficits	[34]

BMSCs, bone marrow stromal cells; hMSCs, human mesenchymal stem cells; i.a., intra-arterial; i.c., intracranial; i.v., intravenous; MCAO, middle cerebral artery occlusion.

Based on existing studies, we conclude that “the earlier the better” is generally the best policy for therapeutic intervention in ischemic stroke. Kawabori *et al.* transplanted BMSCs *via* i.c. after MCAO at either 1 or 4 weeks post-ischemia, and found that both low and high doses of BMSCs at 1 week post-ischemia significantly improved functional recovery, whereas at 4 weeks post-ischemia, functional recovery only occurred at the high dose. In

addition, the number of BMSCs required to achieve the same therapeutic benefit was smaller when transplanted earlier *versus* later[35].

In conclusion, the i.c. xenotransplantation of BMSCs is feasible and has great therapeutic potential. Using this method, BMSCs exhibit long-distance migration along the ischemia penumbra and a low immunologic response within the transplant site. Ischemic stroke creates a more suitable host micro-environment as revealed by increased BMSC xenograft survival and distribution in the ischemic brain where the migratory BMSCs express neuronal or astrocytic markers around the ischemic focus[21]. Immunosuppressive therapy may enhance the survival of grafted BMSCs and reduce the graft-induced immunologic response. However, in the acute phase post-transplantation, BMSCs can survive, migrate, and proliferate without immunosuppression[20].

The i.a. injection directly *via* the homolateral carotid artery appears to be superior to i.c. injection, not only in terms of invasiveness and safety, but also in terms of the survival rate of grafted cells in the ischemia-damaged area. BMSCs delivered i.a. into a 24-h ischemia-reperfusion rat model are distributed throughout the territory of the middle cerebral artery at 14 days after MCAO, ameliorate neurological deficits in adhesive-removal somatosensory function, and facilitate a better mNSS grade[5]. In addition, transient ischemic rats receiving i.a. injection of BMSCs displayed prolonged latency and less reduced amplitude of motor-evoked potentials (MEP) after monopolar stimulation; 40% of the BMSC group had measureable MEP induced by bipolar stimulation, and displayed I wave that was not detected in control group[36].

The i.a. route minimizes the time for cells to target the ischemic lesion along the relevant arteries[37] and seems to be more appropriate for treating transient cerebral ischemia. Many experiments have confirmed that the i.a. injection of BMSCs benefits the functional restoration in transient MCAO[24]. But this remains controversial because of capillary artery microthrombosis, which may increase the mortality in experimental rats.

When a syngeneic suspension of completed BMSCs (hematopoietic and mesenchymal) is injected intra-arterially into rats 2 h after MCAO onset, the vast majority of implanted BMSCs (>95%) are detected within the spleen. At 6 h after administration, the first BMSCs are located in and around the ischemic area, with activated microglia around the implanted BMSCs. Although the number of implanted BMSCs detected in the lesion site varies greatly, it increases gradually in the first 12 h after transplantation and begins to decrease after 24 h due to the phagocytotic activity of surrounding activated microglia. The delivered BMSCs strengthen angiogenesis, neurogenesis, and synaptic plasticity by secreting various growth factors, such as vascular endothelial growth factor (VEGF), basic-fibroblast growth factor (bFGF) and brain-derived neurotrophic factor (BDNF), which may be mediated by activated microglia. In conclusion, at an early stage of ischemic stroke, a small number of i.a. BMSCs can significantly reduce the ultimate lesion size[25].

Injecting BMSCs directly *via* the rat tail vein up-regulates the expression of bone morphogenetic proteins (BMP) 2 and 4, gap junction protein connexin-43, and synaptophysin, and abrogates neurological deficits 2 weeks after injection, and the beneficial effects persist for at least one year[24]. The i.v. delivery route is the least invasive and thus most extensively used in both experimental and clinical settings[38]. Fully considering the increase in the risk of vein thrombosis and cell migration to other organs such as liver and lungs, various attempts have been made to improve therapeutic effects of i.v. cell therapy. MSC infusion *via* two boluses, inhibition of MSC CD49d, and infusion of multi-potent adult progenitor cells and neural stem cells increase the passage of cells across the pulmonary micro-vascular barrier[39].

Compared with i.c. injection, i.v. delivery seems to be relatively easy, less invasive, and enables cells to fully interact with various neurotrophic factors and migratory signals. Furthermore, the i.v. route facilitates BMSC adaptation to adverse circumstances[38], promotes the expression of the anti-apoptotic proteins survivin and Bcl-2, and improves sensory-motor function in rats after ischemic stroke[33].

While i.c. injection is the most precise way to target cells in the peri-lesional area, the i.v. approach appears to be a preferable choice for global cerebral ischemia by reducing neuronal loss and eliciting functional recovery[34].

In another study, BMSCs were immortalized by transfection with the human-telomerase gene (hTERT-MSCs). The i.v. delivery of hTERT-MSCs into cardiac arrest models after transient (2 h) MCAO improved rat

performance in the Morris water maze and treadmill test, and reduced infarct volume. The magnitude of reduction of infarct volume and functional improvement was positively associated with the number of hTERT-MSCs delivered[40].

The i.v. injection of allogenic or heteroallogenic BMSCs into female rats after transient (2 h) MCAO both restored neurological function at 28 days, but no significant difference was found between allogenic and heteroallogenic cell-treated rats[41]. We surmise that the decrease of rejection is associated with immunosuppression in the ischemic brain. In another study, Li *et al*. extended the time of cell transplantation to 1 month after MCAO, and found that i.v. injection of BMSCs at this time point improved functional recovery in the mNSS and in the adhesive-removal somatosensory test[42]. Therefore, the optimal time window for BMSC therapy may be at least 1 month after ischemic stroke.

Accumulating evidence suggests that i.v. application of hMSCs in rats after MCAO increases neurogenesis and angiogenesis, and significantly improves functional recovery[28-30]. In addition, hMSCs can escape immune system surveillance because of the poorly-recognized antigens on the cell surface[30]. However, the comparative therapeutic benefits at different time points are still unclear. BMSCs injected at the onset of MCAO or 6 h later significantly improved behavioral and neurological recovery after six weeks[43]. Similarly, Omori *et al.* infused hMSCs intravenously at multiple time points[44], and found that the greatest therapeutic benefit occurred for hMSCs injected at 6 h after MCAO.

Therapeutic Pre-conditioning of BMSCs for Ischemic Stroke Treatment

Many experiments have confirmed that BMSCs are capable of promoting the regeneration of damaged nerve tissue and ameliorating neurological deficits. The likely BMSC-related therapeutic mechanisms underlying recovery in the ischemic stroke model still need further exploration. Under ischemic conditions, transplanted BMSCs can migrate to damaged brain tissue[38], restore neurological function by differentiating into the neurons[13], and/or inhibit apoptosis and express neurotrophic factors, in addition to stimulating endogenous factors, so as to promote neurogenesis and synaptic plasticity. To achieve a better therapeutic effect, different BMSC pre-conditioning methods have been tested in ischemic stroke[45] (Table 2).

In the early post-treatment phase, BMSCs have a stimulating effect on the expression of cytokines and neurotrophic factors in the ischemic zone[59]; these provide trophic support for vulnerable neurons, particularly in the penumbra. Moreover, intrastriatal delivery of BMSCs transfected with the fibroblast growth factor-2 gene (FGF-2) improves neurological functions and significantly decreases infarct volume in MCAO rats[46]. The improved outcome likely benefits from the neuroprotective or vasodilating effect of FGF-2.

The i.v. infusion of hMSCs with genes for various growth factors or neurotropic factors including BDNF, glial cell-derived neurotrophic factor (GDNF), and VEGF significantly ameliorates the neurological defects in cerebral ischemia. Further, BMSCs modified to enable hyper-secretion of neurotropic factors have a greater therapeutic effects in reducing infarct size and promoting functional recovery than those modified with growth factor genes[49-52]. A positive correlation between the expression levels of modified genes and the therapeutic effect of modified BMSCs has been found[50-52]. The angiopoietin-1 (Ang-1) and VEGF genes are thought to be of critical importance in angiogenesis[60]. The i.v. injection of a combination of Ang-1 and VEGF gene-modified hMSCs (Ang-VEGF-hMSCs) produces greater structural-functional recovery than Ang-hMSCs and VEGF-hMSCs[60].

BMSC-derived neuronal cells (MSDNCs), induced by gene transfection with the Notch intracellular domain and subsequent treatment with bFGF, forskolin, and ciliary neurotrophic factor, were delivered into the focal cerebral infarct on day 7 after MCAO[53], and improved behavioral recovery in the balance beam test, the limb placing test, and the Morris water maze test[53].

However, insufficient nutrition and reactive oxygen species toxicity in ischemic lesions can induce apoptosis in the delivered cells and limit the efficacy of BMSC therapy. Noggin, an antagonist of BMP, regulates the differentiation of stem cells into neurons[61]. The i.v. delivery of Noggin-transfected BMSCs at 6 h after MCAO, significantly promotes neurogenesis in the subventricular zone (SVZ) of rats at 7 days, enhances the BMSC-induced neuroprotective effect, decreases infarct volume, and ameliorates neurological deficits[57]. The coexistence of NGF and Noggin in BMSCs has a synergistic effect on the

Table 2. Therapeutic pre-conditioning of BMSCs for ischemic stroke treatment

Stroke model	Delivery route	Grafted cells	Timing	Results	Ref
Transient MCAO	i.c.	FGF-2/HGF-BMSCs	2 h/24 h	Remarkable decrease of infarct volume, neuro-protective or vasodilating effect of FGF-2 and HGF	[46, 47]
Permanent MCAO	i.c.	G-CSF-BMSCs	7 days	Up-regulated expression of SDF-1α, HGF, and nerve growth factor	[48]
Permanent MCAO	i.v.	BDNF/GDNF/PlGF/ VEGF-hMSCs	6 h/3 h	hMSCs modified to hyper-secrete neurotropic factors decreased infarct size and promoted functional recovery.	[49-52]
Permanent MCAO	i.c.	MSDNCs-bFGF/CNF	7 days	Behavioral functional recovery in beam balance test, limb placing test, and Morris water maze tests	[53]
Barrel cortex ischemic stroke	i.c.	Hypoxic BMSCs	24 h	BMSCs decreased infarct size and restored thalamocortical circuit connection.	[54, 55]
Focal cerebral ischemia	Intranasal	Hypoxic BMSCs	24 h	BMSCs decreased cell death in IBZ and reduced infarct volume. Hypoxic preconditioning of BMSCs enhanced cell-homing and optimized therapeutic efficacy.	[56]
Permanent MCAO	i.v.	Noggin-BMSCs	5 days	Promoted neurogenesis, decreased infarct volume, and ameliorated neurological deficits	[57]
Permanent MCAO	i.v.	CXCR4-rMSCs	24 h	CXCR4 overexpression in BMSCs promoted mobilization and migration, enhanced neuroprotection.	[58]

BDNF, brain-derived neurotrophic factor; BMSCs, bone marrow stromal cells; CXCR4, chemokine (C-X-C motif) receptor 4; FGF-2, fibroblast growth factor-2; G-CSF, granulocyte-colony stimulating factor; GDNF, glial cell-derived neurotrophic factor; HGF, hepatocyte growth factor; hMSCs, human mesenchymal stem cells; IBZ, ischemic boundary zone; i.c., intracranial; i.v., intravenous; MCAO, middle cerebral artery occlusion; MSDNCs, bone marrow stromal cell-derived neuronal cell; PlGF, placental growth factor; rMSCs, rat mesenchymal stem cells; VEGF, vascular endothelial growth factor.

improvement of neurological function and promotion of synaptophysin expression in the ischemic brain. In addition, increased expression of NGF or Noggin in BMSCs is beneficial to brain plasticity[62].

Hypoxic pre-treatment applied to stem cells before transplantation improves the survival rate of the grafted cells[63]. Hypoxic treatment results in up-regulated expression of hypoxia-inducible factor-1α and secretion of trophic/growth factors (BDNF/GDNF/VEGF) by BMSCs, and down-regulates many pro-inflammatory cytokines/chemokines. BMSCs pre-processed with sub-lethal hypoxia (0.5% O_2, H-BMSCs) significantly improve motor function in the rotarod test 15 days after transient (90 min) MCAO in rodents[64].

The barrel cortex ischemic stroke model, characterized by a relatively small infarct volume, and a well-defined ischemic edge and peri-infarct region[54], is often used to evaluate the recovery of ischemia-damaged neuronal circuit connectivity. H-BMSCs (0.5% O_2 for 24 h) delivered at 1 day and 7 days after focal barrel cortex ischemic stroke, promote vascularization and axonal growth, which are attributed to the up-regulated expression of axonal growth-associated protein-43 and accompanied by down-regulated expression of the axonal growth-inhibiting proteins ROCK II and NG2. On day 3 after ischemic stroke, the infarct volume clearly decreases. Six weeks later, the recovery of intracortical activity and partial restoration of thalamocortical circuit connections likely contribute to the improvement in sensorimotor function[55].

The interaction between SDF-1α and its cognate receptor CXCR4 is crucial for the homing and migration of multiple stem-cell types[58, 65]. In the early phase of recovery

from ischemic stroke, CXCR4 expressed by BMSCs can be activated[66]. CXCR4 overexpression in BMSCs promotes their mobilization and migration to the ischemic zone, enhances neuroprotection, and promotes functional recovery[58].

Combination Therapy for Stroke Using Drug Treatment and BMSC Transplantation

BMSC monotherapy is confronted by various challenges, including apoptosis and the mass death of grafted cells. Recent studies have claimed that appropriate application of pharmaceuticals (such as Z-VAD, SDF-1α, and statins) decrease the apoptosis of grafted BMSCs, induce migration into the ischemic area, modulate the expression of protein/cell factors and trophic factors, promote angiogenesis in the ischemic area, and enhance the therapeutic effect of BMSC transplantation.

When injected into the ischemic brain, very few grafted BMSCs survive due to the resultant apoptosis. When BMSCs are co-administered with the cell-permeable inhibitor of caspases, Z-VAD, into rats after transient (2 h) MCAO, the survival rate of BMSCs is enhanced, the apoptotic rate is lowered, and functional recovery is improved[67]. In addition, the i.v. infusion of hMSCs with the nitric oxide donor, (Z)-1-[N-(2-aminoethyl)-N-(2-ammonioethyl) aminio] diazen-1-ium-1,2-diolate (DETA/NONOate), benefits functional recovery in transient (2 h) MCAO rats compared with BMSC monotherapy. Besides, both the number of grafted BMSCs in the SVZ and the expression levels of VEGF and bFGF in the ischemic boundary zone increase. Improved brain plasticity resulting from treatment with hMSCs and DETA/NONOate may contribute to enhanced therapeutic effects and functional improvement[29].

In addition, combined treatment with BMSCs and DETA induces a greater reduction of neurological deficits in rats after transient MCAO compared with BMSC monotherapy[68]. A further investigation revealed that DETA-NONOate increases the expression of CXCR4 and MMP in BMSCs, and promotes BMSC adhesion and migration to mouse brain endothelial cells and astrocytes[68]. In addition, combined i.v. injection of BMSCs with DETA up-regulates angiopoietin-1 and its receptor Tie2, and restores neovascularization, which is regarded as the main therapeutic goal in ischemic stroke[69].

The i.c. co-administration of SDF-1α and BMSCs promotes BMSC migration to the ischemic lesion, increases the density of blood vessels in ischemic cortex, enhances neuronal plasticity, and up-regulates the expression of anti-apoptotic proteins[70]. Sodium ferulate (SF) has a neuroprotective effect *via* its anti-apoptotic and anti-inflammatory properties in ischemic brain. Combined injection of SF with BMSCs further increases the expression of SDF-1α/CXCR4, and improves the homing of BMSCs towards the ischemic area. It has been suggested that SF increases the differentiation of BMSCs into Nestin-positive cells, promotes the recruitment of Nestin-positive cells to the ischemic area, and significantly improves functional recovery[71].

To achieve the optimal therapeutic effect of BMSCs safely, Osanai *et al.* grafted the BMSC-TGP construct (a thermoreversible gelation polymer (TGP) hydrogel) onto the ipsilateral intact neocortex of adult mice at 7 days after MCAO. Neither TGP hydrogel nor inflammation was detected in the brain. Meanwhile, the hydrogel promoted the migration of grafted cells to the cerebral infarct zone as well as the adjacent dorsal neocortex. TGP hydrogel appears to be effective and noninvasive, and therefore has potential applications for BMSC transplantation therapy for various diseases of the central nervous system due to its unique biochemical properties (nontoxic, easily modifiable, immunologically inert, and absorbable)[72].

Lipid-lowering therapy also plays an important role in the prevention and treatment of ischemic stroke. Statins not only lower serum cholesterol and low-density lipoproteins but also prevent thrombosis, protect endothelium, and prevent arteriosclerosis. In addition, combined treatment with simvastatin and BMSCs induces greater improvements of neurological function, which are mainly attributable to the pharmacological effects of simvastatin on the reduction of infarct volume and decrease of brain edema[73].

Clinical Trials of BMSC Transplantation for Ischemic Stroke

Currently, much progress has been made in BMSC therapy for ischemic stroke using animal models. However, the ethical dilemmas of embryonic stem-cell research and the problems associated with the immune response and safety largely have limited their clinical use. In 2005, Bang *et al.*[74] intravenously injected autologous MSCs into patients with

ischemic stroke. For 1 year after symptom onset, patients in the MSC group showed a modified Rankin score, which coincided with improved neuroimaging, and no evident side-effects of MSC infusion were detected. The finding that i.v. administration of autologous MSCs appears to be a feasible and safe therapy for ischemic stroke has fuelled excitement in the neurological community. A critical concern, however, remains because the number of patients in the treatment group was small, and more trials are needed. Lee *et al.* followed the outcomes of patients receiving i.v. delivery of MSCs for up to 5 years. The higher cumulative number of surviving patients in the MSC group at 260 weeks indicates the long-term safety and efficacy of i.v. MSC transplantation in a larger population[75]. In clinical trials, i.c. delivery is considered invasive and results in the physical dysfunction of cells. Four out of 30 patients experienced seizures and subdural hemorrhages after intrastriatal transplantation of BMSCs[76]. Battistella *et al.* transplanted autologous bone marrow-derived mononuclear cells (BMNCs) into patients with MCA ischemic stroke within 90 days after symptom onset (non-acute phase)[77]. Similarly, Moniche *et al.* transplanted BMNCs intra-arterially into patients 5–9 days after ischemic stroke onset (sub-acute phase)[78]. During the following period, patients with i.a. BMNCs showed no worsening in neurological function, and no severe treatment-related adverse events. Their data indicate that the i.a. transplantation of autologous BMNCs in MCA ischemic stroke is feasible and seems to be safe[77, 78]. Recently, Friedrich *et al.* used the same treatment approach at 3–7 days following stroke onset in patients with early or late spontaneous recanalization but with persistent deficits. Satisfactory clinical improvement at 90 days after treatment was achieved by a proportion of the participants, demonstrating the safety and viability of BMNC i.a. infusion for patients with moderate-to-severe acute MCA[79].

Challenges and Prospects

The published data show great promise for BMSC transplantation as a therapeutic strategy for ischemic stroke with unique advantages, including multidirectional differentiation, convenient material acquisition, a high rate of amplification, and the overcoming of many obstacles associated with ethics and immunological rejection. Furthermore, modification of BMSCs with neuroprotective factor- or neurotrophic factor-encoding genes enhances the therapeutic efficacy. Nevertheless, the clinical application of BMSC transplantation is limited, and safety issues must be considered. The fundamental properties of BMSCs and their potential for short- and long-term toxicity need to be determined before they can be widely used in clinical practice. Substantial challenges must be addressed and resolved to advance the use of BMSCs for cerebral ischemia treatment. For example, challenges that remain include: determining the optimal technique for injecting BMSCs; reaching an agreement with the patients on the therapy; timing BMSC transplantation to maximize the therapeutic effect on infarcts as well as determining the appropriate dosage of grafted BMSCs; defining the mechanism of grafted BMSC migration to the ischemic zone and BMSC differentiation; and identifying whether transplanted BMSCs are integrated into neuronal circuits. Studies to answer these critical questions will require close collaborations and interactions among scientists and clinicians.

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