Chapter 11
Bone Marrow Stem Cell Delivery Methods, Routes, Time, Efficacy, and Safety

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Abstract Stem cell transplantation offers an exciting new therapeutic avenue for stroke, as many studies have demonstrated favorable results in animal models with various cell types. Several early phase I and II clinical trials are now underway with promising outcomes. However, cell transplantation for stroke is still in its infancy with many issues that need to be addressed in order to achieve full potential as a therapy. Among the major hurdles for a successful clinical translation is determining the optimal conditions of transplantation for stroke. In this chapter, we review the impact of implanted cell number, delivery sites of cells, and transplantation time on the stroke outcome. In addition, we also discuss the efficacy and safety of bone marrow stem cell transplantation for stroke treatment.

Keywords Transplantation • Stroke • Optimal condition • Time • Cell number

11.1 Introduction

Stem cell transplantation offers an exciting new therapeutic avenue for stroke, as many studies have demonstrated favorable results in animal models with various cell types. Several early phase I and II clinical trials are now underway with promising outcomes. Therefore, the potential therapeutic impact of stem cell transplantation on regeneration of damaged brain tissue opens up enormous possibilities. If successful, millions of stroke survivors with disability may benefit. However, cell transplantation for stroke is still in its infancy with many issues that need to be addressed in order to achieve full potential as a therapy. Among the major hurdles

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K. Jin et al. (eds.), *Bone Marrow Stem Cell Therapy for Stroke*,
DOI 10.1007/978-981-10-2929-5_11

for a successful clinical translation is determining the optimal conditions of transplantation for stroke. As different groups used different protocols or conditions, the results may not compare well with each other, which makes it difficult to determine the best conditions for stem cell therapy following a stroke. The optimal conditions, including the best cell type, the cell number, the timing of transplantation, the route and site of delivery, and the stroke model, are highly important. All of those conditions still need further study. In the following parts, we will summary recent studies, which are associated with BMSC delivery method, time point, efficacy and safety.

11.2 Delivery Methods and Routes

Bone marrow stem cell (BMSC) transplantation is a promising therapy for some kind of diseases like traumatic spinal cord injury (SCI) and degenerative conditions of the central nervous system (CNS). The number of transplanted cells in the brain depends on the effectiveness of the transplantation. BMSC transplantation has been investigated and explored in animal models to determine its therapeutic effects for disorders such as SCI and brain ischemia [1]. BMSCs produce different trophic factors (e.g., brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), nerve growth factor (NGF), and hepatocellular growth factor (HGF)) and cytokines. BMSCs do not only activate endogenous restorative responses such as angiogenesis, synaptogenesis, and neurogenesis, they also have a negative effect on the death of the brain cells in the ischemic boundary zone [2]. Prior to the administration of BMSCs, it's required to culture them for a certain amount of time [3]. BMSCs have the protective potentials to repair the tissue [4]. Also BMSCs have the ability to cross the blood–brain barrier (BBB) and migrate into the brain parenchyma [5]. In order to achieve greater efficacy in terms of neuroprotection, BMSCs should be modified and injected intracerebrally [6]. The neuroprotective effects of BMSCs may result from their behavior as small molecular factories [7], although the basic mechanisms are still not comprehensive. Different routes of administration BMSCs exhibit different effects in treating disorders.

11.2.1 Intravenous and Intra-arterial Routes

When BMSCs are intra-arterially administered, larger numbers of the cell are able to reach their target tissues such as the brain parenchyma as opposed to the intravenous (IV) routes. The cells are thus able to confer their neuroprotective functions efficiently through the intra-arterial (IA) routes. Protective factors from the marrow cells provide the neuroprotection after transplantation. Injection of BMSCs into the ipsilateral carotid artery after transient middle cerebral artery occlusion (MCAO) results in many BMSCs in the ischemic hemisphere [1]. Transplantation of BMSCs after brain ischemia results in up to 21 % of the cells in the MCA territory [2]. Administration of BMSCs after brain ischemia decreases infarct volume and increases transplanted cells in the brain when infused in intravenously [8]. Following

transplantations of BMSCs, analysis of brain sections can be performed by fluorescence photography. BMSCs transplanted by IA routes appear to express larger numbers of PKH26-positive cells in an ischemic hemisphere compared to the IV routes. IA transplantation is an effective route in reducing infarct volumes unlike the IV routes. Rotarod score test of an IA-transplanted BMSCs shows improved and higher motor function than the IV-transplanted ones. BMSCs thus improve functional outcome when administered intra-arterially following ischemia [3, 9]. Reports indicate that adverse effects after IA administration of BMSC are often minimal and insignificant in patients with stroke [10–12]. IA routes however require a cerebral angiography which is an invasive procedure with its associated risk factors such as new strokes. The risk is nevertheless very minimal with research indicating the possibility of a new stroke is about 0.14% and other complications is close to 2% [13]. Other notable risk factors following BMSC transplantation intra-arterially involve embolism and occlusion of brain vessels [14]. IV routes thus might be safer than IA routes to some extent. That's not convinced that IA routes seem to be an effective and a superior route than the IV routes. Further research and validation need to be performed to elucidate that one route is more functional than the other [15]. Stem cell delivery to the injured spinal cord has to overcome several arterial branches to maximize efficacy. Hence, a highly selective and technically challenging cannulation is required. One advantage of intravenous stem cell delivery is comparatively the least invasive approach and has been investigated in several studies [16]. After intravenous injection, cellular homing occurs into the pathological CNS tissues. However, IV route is still less efficient when measured with other approaches such as intra-cerebrospinal fluid (CSF). Additional problems associated with intravenous stem cell delivery include reliance on injury-mediated opening of the BBB to allow cell access to the CNS parenchyma (or the need for additional drugs such as lipopolysaccharide to open the BBB) [17]. First-pass effects affect BMSCs and trap them in extra tissues such as the liver and lungs exposing them to longer periods of immunity and reticuloendothelial cells after injection into the bloodstream. Very few to no cells usually present within the injured spinal segments that receive BMSCs intravenously indicates that the effectiveness of cell therapy might not be necessarily related to the number of cells reaching the brain parenchyma [18].

11.2.2 Intrathecal/Lumbar Puncture Routes

Lumbar puncture (LP) is a minimally invasive way of cell and drug delivery, and BMSC may be well suited for LP transplantation because of their responsiveness to signals from the injured CNS [17]. Clinical and theoretical studies show that LP delivery of stem cells is extremely attractive. LP is performed at the L3–4 level, far away from the cervical or thoracic spinal cord, which is the region most commonly effected by SCI. This makes LP delivery of stem cells relatively safe and unlikely to worsen compromised patients as a direct result of the intervention. Stem cells can be injected directly into the lesion site; however, the additional trauma from

intramedullary transplantation can further compromise injured tissue and impede clinical outcomes. A potential and effective alternative is intrathecal transplantation via LP [19]. Additional advantages of LP delivery are related to several factors: (1) they are far more superior to IV route because they are injected directly into the CSF without encountering the BBB; (2) the CSF transports the cells to injured tissues without encountering first effect degradation by the liver or lungs; and (3) because the transplanted cells are delivered away from the hostile environment of the injured tissue, they are given a greater opportunity to survive and migrate to the injured site. Cell transplantation via LP may be relevant for conditions such as multiple sclerosis with widely disseminated lesions making intramedullary transplantation impractical [20]. Neuronal progenitor cells provide neuroprotective functions for injured spinal cord after subacute transplantation directly into the cord [21]. Proliferation of BMSC occurs both in injured and uninjured spinal cord after LP transplantation during the early stages of the transplantation; however, the number of proliferating cells decreases with time. LP-transplanted BMSCs are distributed in the intrathecal space, along the length of the spinal cord, and a few will migrate to the lesion cavity. This implies that BMSC can reach the injured spinal cord using minimally invasive method of LP injection of cells into the lumbar intrathecal space. Prior to transplantation of the cells, extensive incision and disruption of the dura need to be done to allow for transplanted cells to migrate freely into the injured spinal cord [22].

Swelling of the injured cord together with the meningeal reaction causes intrathecally injected cells to be attracted to the cord parenchyma. Collagenous matrix could be implanted to serve as an adhesive substrate for the cells to adhere and attach. The substrate also serves as a barrier for the subarachnoid space to prevent the passage of the intrathecal cells [23].

11.2.3 Cerebral Injections

A major unresolved problem in the context of SCI is the delivery of cells to an already compromised spinal cord without causing further damage. Most investigators have undertaken direct injection into the injured spinal [24]. Although this is acceptable in animal experiments, its extrapolation to humans may be difficult because a major neurosurgical operation will be required. This difficulty in translation will limit clinical trials, at least initially, to patients with complete SCIs in whom further deterioration cannot occur but in whom significant benefit from transplantation therapies is also least likely [25]. Another problem associated with direct parenchymal injections is the likelihood of damaging spared spinal tissues with the injecting needle. It is a well-known principle of neurosurgery that injured tissues do not tolerate operative manipulation as well as normal tissues, because of the presence of edema, altered blood flow, and injury-related cytokines. Finally, direct injection of cells into the parenchyma does not allow suitable delivery of multiple therapeutic doses because of its invasive nature and because injecting cells into multifocal diseases presents many logistical and technical challenges. The direct delivery of stem cells into the CSF has also been explored [26], and intraventricular

injection has been the favored delivery method [27]. This technique, however, is too invasive for clinical applicability, which makes its transplantation challenging. Investigators have demonstrated that neurosphere-derived stem cells delivered into the ventricular CSF can reach the injured tissue in a spinal contusion model [28]. BMSCs are more appropriate because of their evidenced therapeutic effect, their availability, and the possibility of an autologous model in humans. Injection of cells into the lumbar CSF via an indwelling cannula has been shown to be effective for delivering embryonic germ cell derivatives. Considerably more cells will be detected in the injured tissues after both intrathecal and intraventricular delivery. Transplanting cells into the CSF leads to more successful grafting when injection is via an intrathecal or intraventricular rather than intravenous route. The number of cells within the injured spinal cord tissues increased with passage of time. Few cells are present at the early days after transplantation; however, many more cells will be recorded as the time increases after transplantation.

11.3 Effective Times for BMSC Transplantation

Bone marrow stem cell transplantation has over the years been a promising field for the treatment of various disorders like ischemic stroke, hematologic diseases, cardiac disorders like cardiac infarctions, etc. The efficacy of treatment not only depends on the route of cells but also on the cell dose and the time of delivery [29]. For example, de Vasconcelos Dos Santos et al. highlighted the benefit of using BMSCs in the treatment of a thermocoagulation-induced ischemic rat model at different therapeutic windows. They concluded in their experiment that BMSCs might be an efficient treatment protocol for stroke only in the acute/subacute phase of the disease since they were unable to decrease glial scarring significantly [30]. Another example was a recent study which clearly showed that IA administration of 1×10^7 BMSCs immediately after reperfusion is much more effective in delivering BMSCs to the brain than IV administration. In addition, the larger number BMSCs are transplanted in the brain during the early stage of reperfusion, the better protective effect may be presented. The study therefore suggested further understanding into the dose–response influence and therapeutic time window for efficient BMSC delivery to the ischemic site [31].

BMSC transplantation like any other treatment option has an optimum time to which to get adequate outcome. There is therefore the need to optimize treatment by taking advantage of the best time for optimum outcome and reduce disease progress. During ischemia, a series of inflammatory response is initiated which is mediated by many transcription factors of which nuclear factor-kB is a key factor. When hypoxia or ischemia occurs, a cascade of signal transductions is triggered, causing nuclear factor-kB inhibitor IkB phosphorylation degradation and activation of nuclear factor-kB to enter nuclei and stimulate target gene transcription. These processes eventually trigger a positive feedback which leads to an overwhelming inflammatory response. This secondary inflammatory response if not controlled will

accelerate and cause further cerebral ischemia/reperfusion injury. A study performed recently indicated that nuclear factor-kB DNA-binding activity is exponentially enhanced within 6–12 h after ischemia, whereas there is gradual decrease between 24 and 72 h. This indicates that NF-kB translocation occurs in a time-dependent manner after cerebral ischemia [32].

In an experiment to test the hypothesis that IV administration of BMSCs could lead to improvement of functional recovery after MCAO for 45 min in the rat and to determine specific time windows for efficacy. Iihoshi et al. injected rats intravenously with transfected mononuclear cells at 3, 6, 12, 24, and 72 h after MCAO. The ischemic lesion was histologically analyzed at 14 days. It was noted that there was no lesion detected at 3 h transplantation after lesion induction. Lesions were however detected from 6 h post-lesion group and progressively increase at times 12, 24, and 72 h. Infused LacZ(+) bone marrow cells are implanted extensively in and around the ischemic site, with immunohistochemistry studies indicating some amount of differentiation of neuronal and glial cells. Behavioral testing (Morris water maze and treadmill stress test) also indicated improved functional recovery in the transplanted group. These findings further stress the need to intervene as fast as possible and also suggest that IV administration of autologous mononuclear cells from the bone marrow could help improve functional outcome [3]. Other experiments suggest other time windows. Of notable example is the administration of allogeneic human umbilical cord blood MSCs (hUCB-MSC) by LP 3 days after stroke, which was stated to be a valuable method for efficient cell delivery and therapy in stroke model in rats [33, 34].

11.4 Efficacy and Safety of BMSC Transplantation

According to therapy purpose, proper BMSC delivery method should be selected. The delivery efficacy and safety are two critical factors that determine the application of delivery method. When comparing those different delivery methods, every route has some apparently advantages and shortages.

Using IV infusion to deliver BMSC is the simplest and safest method. In animal models, engraftment was demonstrated [35]. However, IV infusion has low cell retention rate; the efficacy is pretty low. Also, BMSC cannot diffuse to specific sites for therapeutic effect. The amount of BMSC arrive the target organ may not efficiently repair the primary injury by differentiation. But, studies demonstrated that recovery improved after BMSC administration should be partially owed to the inflammation milieu improvement by trophic factors and inflammation factors, which are secreted by transplanted BMSC. Comparing to IV infusion, local IA infusion has higher efficacy. The cell distribution after IA infusion of BMSC is much better than IV infusion [36]. IA infusion of BMSC can be applied for heart diseases. IA infusion has some shortage, which may cause microembolism or ischemia during infusion. And sometimes these shortages are fatal. Direct route still is the highest efficacy delivery method, which could deliver a maximum amount of cells to

intended area [37]. Safety would be essential for patients; due to the big invasion, the direct route is limited to some certain situation. LP route is a novel minimally invasive method for delivery of BMSC, which can be applied for many kinds of CNS disease. The important thing is that researches already have demonstrated that BMSC could pass through BBB after LP administration [38, 39]. LP route delivered BMSC able to survive and accumulate and can exhibit the function therapy in CNS disease [22, 40]. The problem is the efficacy of LP still kind of low. Therefore, optimal transplantation technique should be developed to serve maximally safe and efficacy results.

In spite of the restriction of the delivery method, there still have some methods to improving delivery efficacy. Preconditions could improve the survival of BMSC, which including hypoxia and pharmacological treatment. In myocardial infarction model, hypoxia preconditioning can increase the expression of pro-survival and proangiogenic factors including hypoxia-inducible factor 1, angiopoietin-1, vascular endothelial growth factor and its receptor, Flk-1, erythropoietin, Bcl-2, and Bcl-xL. Cell death of hypoxic stem cells and caspase-3 activation in these cells were significantly lower. Transplantation of hypoxic BMSCs after myocardial infarction results in an increase in angiogenesis, as well as enhanced morphologic and functional benefits of stem cell therapy [41]. Indeed, in intracerebral hemorrhage (ICH) model, BMSCs pretreated with hypoxia preconditioning can significantly improve behavioral performance, and increase neurogenesis compared with the vehicle group after ICH [42]. Alternatively, many kinds of pharmacological treatment also could enhance mesenchymal stem cell survival. Trimetazidine (TMZ) preconditioning increases the survival rate of BMSCs through upregulation of HIF1-α in rat myocardial injury model [43]. Hypoxia-inducible factor 1-α (HIF1-α) prolyl hydroxylase inhibitor dimethyloxalylglycine (DMOG) also can upregulate expression of survival and angiogenic factors including HIF1-α, vascular endothelial growth factor, glucose transporter 1, and phospho-Akt, which enhance BMSC survival and therapeutic efficacy after transplantation [44]. Noiseux et al. demonstrated that oxytocin treatment can evoke MSC protection through both intrinsic pathways and secretion of cytoprotective factors [45]. TGF-α stimulates MSC VEGF production in part via a p38 MAPK-dependent mechanism, and preconditioning MSCs with TGF-α could enhance their ability to protect myocardium injury [46]. Tadalafil could increase Bcl2/Bax during the early phase and transcriptional upregulation of PKG-I by STAT3 during the late phase which promotes stem cell protection against ischemic injury [47].

In clinical trials, the efficacy and safety of BMSC transplant for many kinds of diseases have been studied. In stroke patients, the BMSC treatment safety appeared to be safe up to 1 year [48–50]. No significant abnormal EEG/seizures are observed in those patients. Also BMSC transplant treatment in animal stroke model indicated that it has beneficial effects compared to controls [51]. There is another study published by Prasad et al. which showed that intravenous infusion of BMSC doesn't have beneficial effects of treatment on stroke outcome [50]. It is not possible to evaluate efficacy outcome as only one randomized controlled study was available. There still have 15 ongoing clinical trials in phase I or II [52]. After these trials fin-

ish, we can gain more insights into the therapeutic potential of BMSC transplant. In diabetes mellitus, study showed that stem cell transplantation can be a safe and effective approach for therapy [53]. In degenerative diseases of the retina, initial data from early stage clinical trials suggest that short-term safety objectives can be met [54]. However, the question of efficacy will require additional time and testing to be adequately resolved. In spinal cord injury, based on short–medium terms following up, stem cell transplantation appears to be safe and valid in patients and more effective in chronic and complete injury [55]. Nonetheless, prospective, randomized trials in larger cohorts are still needed. In acute myocardial infarction, there is insufficient evidence for a beneficial effect of cell therapy for patients [56]. Further adequately powered trials are needed, and until then the efficacy of this intervention remains unproven.

In summary, base on different disease characters, we can select proper delivery method to increase the safety and efficacy. Preconditions via hypoxia or pharmacological treatments also can improve BMSC survival and enhance the efficacy. Indeed, abundance of researches had demonstrated that BMSCs have beneficial effect on many kinds of disease models. But, for clinical application, the effects of BMSC still need to be confirmed in the following clinical trials. BMSC transplantation therapy is a promising approach for curing so many difficult diseases.

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