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Mesenchymal stem cell-based therapy for ischemic stroke

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Abstract

Ischemic stroke represents a major, worldwide health burden with increasing incidence. Patients affected by ischemic strokes currently have few clinically approved treatment options available. Most currently approved treatments for ischemic stroke have narrow therapeutic windows, severely limiting the number of patients able to be treated. Mesenchymal stem cells represent a promising novel treatment for ischemic stroke. Numerous studies have demonstrated that mesenchymal stem cells functionally improve outcomes in rodent models of ischemic stroke. Recent studies have also shown that exosomes secreted by mesenchymal stem cells mediate much of this effect. In the present review, we summarize the current literature on the use of mesenchymal stem cells to treat ischemic stroke. Further studies investigating the mechanisms underlying mesenchymal stem cells tissue healing effects are warranted and would be of benefit to the field.

Keywords: Mesenchymal stem cells, Exosomes, Ischemic stroke

Background

Stroke is the second leading cause of death and its prevalence is increasing [1]. Stroke can be classified into two types, ischemic and hemorrhagic, of which the former comprises up to 80 % of all cases [2]. Ischemic stroke occurs when blood flow decreases in the cerebrum as a result of an obstruction, such as an embolism or thrombus [3]. Currently, the only approved treatment for ischemic stroke is tissue plasminogen activator (tPA) [4]. However, tPA has a narrow therapeutic window of only 4.5 h from the onset of symptom [5]. Consequently, most stroke patients don't qualify for this treatment and would greatly benefit from the development of novel treatments that have an expanded therapeutic window [5].

Adult stem cell-based therapies, such as mesenchymal stem cells (MSCs) have emerged as a promising approach for the treatment of ischemic stroke [6]. MSCs are good candidates for the treatment of stroke as they are easily obtained and have a strong safety profile [7]. MSCs have demonstrated beneficial effects in improving functional outcome through mechanisms implicated in brain plasticity such as neurogenesis, axonal sprouting, and angiogenesis [6].

In this review, we summarize the current literature on MSCs and their potential use as a therapeutic in cases of ischemic stroke.

Review

Phenotype of MSCs

MSCs are adult multipotent cells which can differentiate into osteo, adipo and chondro lineages [8]. MSCs can be isolated from bone marrow, umbilical cord and adipose tissue [9]. MSCs express the mesenchymal markers CD105, CD90, and CD73 but express few HLA class I and no HLA class II molecules, allowing them to evade allogeneic immune response, making them well suited for allogenic use [10].

MSC mediate tissue healing in damaged organs including ischemic stroke, myocardial infarction and liver injury [11]. MSC activate endogenous cellular repair

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programs by releasing various secretory proteins such as fibroblast growth factor, epidermal growth factor, insulin-like growth factor and monocyte chemoattractant protein-1 [12]. MSCs have also been shown to induce angiogenesis and vascular remodeling via factors such as vascular endothelial growth factor, angiopoietins and hepatocyte growth factor [13]. Additionally, MSCs secrete IL-10, IL-6 and nitric oxide which induce a localized anti-inflammatory state, thereby facilitating the healing of damaged tissue [14].

Several studies demonstrate that small cellularly secreted vesicles called exosomes mediate much of MSCs' tissue healing capabilities [15–28]. MSC derived exosomes are internalized by target cells and transfer proteins, RNA, lipids and metabolites [29]. Our recent study determined that ex-vivo expanded MSCs substantially increase their secretion of exosomes upon exposure to in vivo-like conditions and that these exosomes contain a diverse profile of prosurvival and angiogenic proteins [30].

Studies have demonstrated that MSCs are immunomodulatory and are capable of reducing pathogenic inflammation [31]. MSCs can exert profound immunosuppression both in vitro and in vivo by inhibiting the proliferation of T-cells, natural killer cells, and dendritic cells [32]. MSC have also been reported to induce proliferation of immune suppressive Treg cells, at least in part by inducing the differentiation of monocytes towards resident M2 macrophages [33].

MSCs in the treatment of ischemic stroke

Ischemic stroke is a major cause of death and disability in the aged population [34]. During cerebral infarction, transplanted MSCs migrate to areas of damage and mediate tissue healing [35]. MSCs induce angiogenesis, neurogenesis and neurite outgrowth in the surrounding endogenous tissue through the secretion of neuroprotective factors [6, 24, 36]. MSC have generally been injected intracranially or intravascularly [6]. Some evidence suggests that intravascular MSC administration after stroke may be a viable alternative to intracranial transplantation, but more work in this area is needed before definitive statements can be made [37]. However, intravascular delivery may be better for larger lesions as it could lead to a wider distribution of transplanted cells around lesions than intracranial delivery, but also potentially dilutes out the therapeutic effect across a larger volume.

Numerous studies have reported favorable outcomes in immune-competent ischemic stroke rodent models upon treatment with MSCs (Table 1) [38–57]. Many, but not all, of these studies reported MSCs reduced infarct size and induced functional recovery as reported by lessening of motor deficits or special learning as measured by the radial maze test [38–57]. Many of these studies report a lowering of deficits as assessed by the composite modified neurological severity score (mNSS), while others demonstrated reduced inflammation and apoptosis, as well as increased neurite outgrowth and plasticity [38–57]. Interestingly, recent studies have also determined that exosomes secreted by MSCs are capable of inducing functional recovery in models of ischemic stroke [24, 41, 47, 55, 58].

MSCs, their mechanism of action and safety profile

While not fully understood, postulated mechanisms of actions proposed to account for therapeutic effects of MSCs include cell replacement, growth factor secretion, and biobridge formation [59]. Stroke therapeutics have been categorized as 'neuroprotective' for the acute phase or 'neuroregenerative' for the subacute and chronic stages of stroke [59]. The acute treatment in stroke is relegated to tPA and other drugs that are designed to maintain structure and functionality of the blood vessels. The subacute (several hours to a few days) and chronic (several days, to weeks, months, and even years) phases are the targeted window for MSC transplant therapy. For the subacute phase, MSC transplantation has been shown to abrogate the early secondary cell death responses associated with stroke, such as dampening the oxidative stress, inflammation, mitochondrial impairment, and apoptosis [60]. On the other hand, MSC treatment in the chronic phase has been demonstrated to trigger brain remodeling via angiogenesis, vasculogenesis, neurogenesis, and synaptogenesis [61]. The minimally invasive intravenous or intra-arterial delivery of stem cells has been the preferred choice for the subacute phase due to an already injured brain produced by the primary ischemic insult, combined with chemoattractants that can guide migration of MSCs from the periphery to the brain. The direct intracerebral implantation of stem cells to the peri-infarct region is utilized for the chronic phase with the stroke brain more tolerant of an invasive treatment procedure, but also because of tapered levels of chemoattractants [62]. Direct transplantation was initially examined in chronic stroke patients using neural progenitor cells (NT2N) [63] and in recent years using Notch-induced bone marrow cells (SB-623) [64], with subsequent clinical trials employing intravenous and intra-arterial administration of MSCs in subacute stroke patients [65, 66].

Cell therapy for stroke has tested several types of transplantable cells in the laboratory, with a few reaching clinical trials, such as fetal cells, NT2N cells, CTX0E3, embryonic stem cells, neural stem/progenitor cells, umbilical cord blood, amnion, adipose, and induced pluripotent stem cells [67–72]. Compared to these other stem cells, MSCs have established a solid safety profile in other disease indications, providing the basis for on-going clinical trials to explore MSCs and their cell subpopulations

[73, 74]. As noted above, MSCs have been transplanted intracerebrally and peripherally [73, 75–78], with encouraging pilot studies reporting safety, but efficacy remains to be fully assessed [74].

Translational challenges of MSC therapy for stroke

Recent clinical trials on transplantation of MSCs have shown their safety in stroke [75, 79–81]. In addition to the small number of patients enrolled in these clinical trials, the translation of laboratory protocols for clinical transplant regimens has been marred with major discrepancies including the lack of well-defined release criteria of the donor cells, varying timing, cell dose and route of transplant intervention, altogether deviating from the established preclinical readouts. In particular, many of the clinical trials were not performed along the guidelines of Stem cell Therapeutics as an Emerging Paradigm for Stroke or STEPS lab-to-clinic translational guidelines [82]. The recommended translational research approach is to use at least two models of stroke using small animals (rodents). Any unanswered issues related to safety and efficacy, as well as insights into mechanisms of action, need to be pursued using a large animal model (non-human primates). A rigorous preclinical testing, as recommended by the STEPS guidelines will increase the likelihood of success of future clinical trials of MSC transplant therapy for stroke.

Conclusions

Although numerous studies have demonstrated that MSCs facilitate tissue healing and functional recovery in rodent models of ischemic stroke, several outstanding issues in the field warrant further investigation. The underlying mechanisms by which MSCs respond to their environmental niche upon injection healing is poorly understood [83]. MSCs generally have a relatively short half-life which limits their ability to heal damaged tissue [84]. A major goal for the field should be to develop

Table 1 Studies demonstrating the efficacy of MSC-based therapies for the treatment of ischemic stroke in rodent models

Author	Type of Cells	Stroke model	Delivery	Effect	Histology	Outcomes
Brenneman, M	BM-MSC (Rat)	CCAO/MCAO	24 h	Y	TCC, TUNEL, DAPI, Fluorescein	Decreased infarct
Chen J	BM-MSC (Rat)	MCAO	24 h	Y	H&E, Y chr, TUNEL	Increased rotarod, adhesive
Chen, JR	BM-MSCs (Rat)	MCAO	Immediate	Y	Nissel, GFAP, GalC, MAP2, Tuj1, BrdU	Decreased infarct
Doeppner TR	BM MSC & Exosomes (Human)	MCAO	Days 1,3,5	Y	cresyl violet, Neun, BrdU	Increase rotarod, tightrope
Goldmacher, GV	BM-MSCs (rat)	MCAO	Immediate	Y	TTC, HNA, GFAP, CD11	Decreased nMSS
Fernandez, M	Adipose-MSC (Human, Rat)	Permanent MCAO	30 mins	Y	H&E, TUNEL, GFAP. VEGF, SYP, DAPI	Decreased cell death
Honma, T	MCS-Telomerase (Human)	MCAO	12 h	Y	H&E, TTC. Beta-gal, NeuN, GFAP	Decreased infarct, inflammation
Koh, SH	UC-MSC (Human)	MCAO	2 weeks	Y	Neun, SNE, GFAP, nestin	Decreased nMSS
Li Y	BM-MSC (Human)	MCAO	1 day	Y	H&E, NeuN, MAP-2, GFAP, vWF, TUNEL	Decreased mNSS
Lim, JY	UC-MSC (Human)	MCAO	72 h	Y	TTC, NeuN, GFAP, DAPI, TUNEL	Decreased infarct
Liu, N	BM-MSC-SVV (Rat)	MCAO	26 h	Y	TCC, NeuN, GFP	Decreased infarct
Nomura, T	BM-MSC-BDNF (Human)	MCAO	6 h	Y	TCC, Beta-gal, NeuN, GFAP	Decreased infarct
Quittet MS	BM-MSC-PAM-VEGF (Rat)	MCAO	24 h	N	BrdU, NeuN, GFAP, CASP2, DCX, Ki67	No Difference
Wei, L	BM-MSC (Rat)	MCAO	24 h	Y	BrdU, NeuN, MAP2, GFAP, Tuj1, Iba-1	Increased rotarod
Yamauchi T	BM-MSC (Human)	Permanent MCAO	7 days	Y	Tuj-1, NeuN, GFAP	Increased rotarod, radial maze
Yang C	BM-MSC-HIF1a (Rat)	MCAO	6 h	Y	TTC, CD105	Decreased infarct, nMSS
Toyoshima, A	BM-MSC (Rat)	MCAO	24 h	Y	DAPI, Q-Tracker, TCC	Decreased infarct, nMSS
Xin. H	BM-MSC (Rat)	MCAO	24 h	Y	BDA-DAB, NF-200, SYP	Decreased adhesive, foot-fault
Xin. H	BM-MSC (Mouse)	MCAO	24 h	Y	Nissl, Luxol, SYP, Apo-TAG	Increased neurites, plasticity
Lowrance, SA	BM-MSC (Rat)	MCAO	7 days	Y	Hoescht, GFAP	Decreased SORT

strategies augment the half-life of MSCs upon injection into affected tissue [10]. Hypoxic preconditioning has garnered some beneficial effects in this regard, but more investigation is needed [8].

The molecular mechanisms underlying MSCs therapeutic effects are also poorly understood at present. More detailed mechanistic studies of MSCs' therapeutics effects are warranted and sorely needed. These endeavors would be greatly rewarding in a field where more and more labs are attempting to genetically engineer MSCs with enhanced therapeutic profiles [9]. In addition, MSC secreted exosomes is a field that merits continued exploration, as the discovery of these vesicles has given us the profound insight that the sheer variety of communication signals MSC use to mediate tissue is likely orders of magnitudes higher than previously expected. Indeed, the field has only recently begun to investigate the protein, RNA, lipid and metabolite cargo exosomes transport from MSCs to neighboring cells.

Abbreviations
mNSS: Modified neurological severity score; MSCs: Mesenchymal stem cells; tPA: Tissue plasminogen activator

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Availability of data and materials
This paper is a review article. Referred literature in this paper has been listed in the references part. The datasets supporting the conclusions of this article are available online by searching the PubMed. Some original points in this article come from the laboratory and clinical practice in our research centers and the authors' experiences.

Authors' contributions
JDA: Conception and design, collection and/or assembly of data and interpretation, manuscript writing, final approval of manuscript; HJJ: Collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; CSG: manuscript writing, final approval of manuscript; MV Collection and/or assembly of data; MTP: Collection and/or assembly of data, final approval of manuscript; CSB: Collection and/or assembly of data, final approval of manuscript; MSM: Collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; RLB: Collection and/or assembly of data, data analysis and interpretation, final approval of manuscript; GB: Conception and design, manuscript writing, final approval of manuscript; KDF: Manuscript writing, final approval of manuscript; BF: Conception and design, manuscript writing, final approval of manuscript; FC: Conception and design, manuscript writing, final approval of manuscript; SEA: Conception and design, manuscript writing, final approval of manuscript JL: Financial support, manuscript writing, final approval of manuscript; JAN: Financial support, manuscript writing, final approval of manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
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