Review Article

Therapeutic approach of stem cell transplantation for neonatal white matter injury

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Abstract: The white matter in brain are mainly composed of oligodendrocytes and myelinated axons, and are important for the transmission of neural signals in central nervous system. White matter injury (WMI) is a leading cause of neurocognitive deficits in premature infants as the oligodendrocytes progenitors are easily attacked by hypoxia-ischemia (HI). Various clinical methods are used to treat this disease, while none of them could reverse the sequelae of WMI completely. With the development of stem cell technology, stem cell therapy has attracted huge interest as a novel treatment for WMI. A number of investigations have demonstrated the potential therapeutic effects of stem cell transplantation on WMI. Different types of stem cells have also been used by many researchers to test the therapeutic effect on WMI animal models, such as neural stem cells (NSCs), glial progenitor cells, mesenchymal stem cells (MSCs). In addition, some clinical trials have been conducted. Evidence suggests that transplantation of these stem cells into animals contributes to functional recovery after experimental WMI. The mechanisms of stem cells therapy may include differentiation into neurons and glial cells to replace lost cells, activation of endogenous NSC regeneration, and promotion of the release of neurotrophins. In this review, we summarized effects of different types of stem cells transplantation, the underlying mechanisms, the unsolved problems and concerns before clinical trials and transformation of stem cell therapy for WMI.

Keywords: White matter injury (WMI); stem cell transplant; neonatal

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Introduction

WMI is mainly caused by demyelination of central neuronal cells, which can lead to motor, visual and cognitive disorders. Oligodendrocytes are the only cell type to form myelination. Late oligodendrocyte progenitors at GW24-32 in humans are susceptible to hypoxia-ischemia (HI) or inflammation, therefore resulting in demyelination in periventricular, subcortical and callosal white matters (1). There are some guidelines for WMI treatments, basically supporting or symptomatic treatments. Erythropoietin and hypothermia are also used for treating WMI (2). These treatments can promote neurons regeneration, reduce neuronal loss and axonal injury, but none of them can repair myelin loss. There are growing evidences from clinical and pre-clinical studies that stem/progenitor cells have multiple roles in treating neurological diseases including WMI. Stem cells are undifferentiated cells that include two broad categories: embryonic stem cells (ESCs)/induced pluripotent stem cells (iPSCs) and adult stem cells. ESCs are

isolated from the inner cells mass of blastocysts, which have the capacity of self-renewal and multilineage differentiation. iPSCs are a type of pluripotent stem cells that can be obtained by reprogrammed somatic cells and have similar properties to ESCs (3). Adult stem cells can be obtained from many tissues, such as brain, adipose and bone marrow. Depending on their tissue sources, they can be classified as NSCs, MSCs, hematopoietic stem cells, etc., and they all have been widely used in clinical and pre-clinical researches. As early as 1981, NSCs isolated from animal brain tissue were transplanted for treating neurological diseases, after that, human fetal brain tissue was used (4). In 1997, researchers transplanted glia cells which were obtained from the spinal cord of a normal dog into a neonatal or adult canine with myelin mutant. They found that the graft could survive for a long term and form myelin sheath in the transplantation site (5). After that, many types of cells such as NSCs, MSCs, and oligodendrocyte progenitor cells (OPCs) isolated from primary tissue or derived from ESCs/iPSCs were used for transplantation to treat encephalopathy of prematurity (6). In recent years, with the development of stem cell technology, stem cell transplantation has become a potential therapeutic approach for many neurological defects including WMI. Different types of stem cells proved to be therapeutic in WMI. However, the clinical application of stem cell-based therapy for WMI still faces many challenges, such as immune rejection and limited effect (7). This paper reviewed the progresses and the challenges of stem cell therapy for WMI.

WMI pathology

WMI in preterm infants is mainly caused by perinatal HI, and could lead to a long-term neurologic disability or even death. With the development of perinatology, the survival rate of premature infants increases, accompanied by the increasing incidence of WMI. In the rat model of HI, it was found that the mechanism of white matter damage in premature infants caused by HI was related to the maturation dependent vulnerability of oligodendrocytes. White matter maturation in rats/mice are at postnatal day (PND) 3–5, which corresponds to 24–30 gestational week (GW24-30) in humans. During this period, oligodendrocytes are at the late progenitor ($O4^+/O1^-$) stage and are highly susceptible to HI (8). Late oligodendrocyte progenitors are the main apoptosis cells in the oligodendrocyte lineage when HI occurs. Early OPCs ($NG2^+/O4^-$) and mature oligodendrocytes (MBP^+) are more tolerant to HI. Besides, some late oligodendrocyte progenitors which survived from HI damage, will go through an accelerated differentiation process and become activated oligodendrocytes. However these activated oligodendrocytes have lost the ability of myelination, resulting in demyelinating lesions in the white matter (9). Next, immaturity of the cerebral blood supply in the deep periventricular regions such as basal ganglia of the brain, making it vulnerable to cerebral ischemia. When hemodynamics change, the underdeveloped cerebral vascular system cannot steady blood flow, thus aggravating the vulnerability of white matter to HI. In addition, free radical formation and excitotoxicity of glutamate also contribute to WMI (9).

NSCs transplant

NSCs are pluripotent stem cells with the potential of self-renewal and multi-differentiation. It can be obtained from the fetal/adult brain tissue, ESCs/iPSCs, or direct reprogrammed by astrocytes (10)/fibroblasts (11). NSCs are the most commonly used cell type for WMI treatment because of its potential to differentiate into neurons and glial cells *in vivo* and in vitro. In some primitive studies, mouse primary NSCs were isolated for cell transplantation to treat WMI. Rumajogee *et al.* (12) transplanted adult NSCs isolated from transgenic adult mice expressing yellow fluorescent protein (YFP) into the corpus callosum (CC) of HI mice at PND21. Treated mice in this study demonstrated repair of lesioned structures by histology and magnetic resonance imaging (MRI), and remyelination of the CC by endogenous oligodendrocytes. Behaviors such as cylinder and Cat-Walk tests were qualitatively improved in transplanted mice. Researchers found that NSCs derived from human ESCs obtained similar therapeutic effects (13). Besides, Daadi *et al.* (14) found that the axon of transplanted cells can grow into the lesion site. This suggests that the transplanted NSCs have the potential of integrating into the host's neural circuits, but it requires more electrophysiology evidence. They also observed that neurogenesis, glial regeneration, and neurotrophic support related gene expression upregulated by microarray analysis. These studies have found that NSCs can improve the outcome of WMI caused by HI both in structure and function. As for myelination repair, the mechanism of NSCs transplantation for remyelination is mainly to promote endogenous myelination, rather than to directly differentiate into oligodendrocytes to replace

the lost myelin sheath, because these transplanted NSCs are hardly differentiate into oligodendrocytes in a default environment (12). Recently, due to the development of gene editing technology, genetically modified NSCs have been used to improve the therapeutic effect of NSCs. Tian *et al.* (15) strengthened therapeutic effects of NSCs by overexpressing leukemia inhibitory factor (LIF), which has neuroprotective effect on NSCs. They found that LIF-NSCs could reduce neuron apoptosis *in vitro*. *In vivo*, LIF-NSC reduced the infarction area, increased nerve and glia cell regeneration. In the future, gene editing technology and stem cell therapy will be combined to optimize the therapeutic effect of NSCs.

Mesenchymal stem cells (MSCs) transplant

MSCs are pluripotent stem cells that obtained from tissue such as umbilical cord blood (UCB), bone marrow, adipose tissue or placenta. Under certain culture conditions, they can differentiate into many cell types include neurons and glia cells. Studies have confirmed that in the sheep model of WMI, white matter damage caused by HI was reduced after transplantation of UCB-derived MSCs via resisting inflammatory and modulating immune response (16). van Velthoven *et al.* (17) established the animal model of WMI in PND9 rats, and then transplanted bone marrow derived MSCs into the lateral ventricle of WMI rats. They found that the loss of neurons and oligodendrocytes were significantly reduced, and the motor function of the rats in the transplantation group was improved significantly. In addition, after bone marrow derived MSCs being transplanted for 2 weeks, the proliferated neurons ($BrdU^+/NeuN^+$), oligodendrocytes ($BrdU^+/Olig2^+$) and astrocyte ($BrdU^+/S100b^+$) in the HI + MSC-treated animals were increased, while the proliferated microglial cells ($BrdU^+/Iba1^+$) were decreased compared with HI animals. These studies demonstrated that MSCs in the WMI animal models promoted the regeneration of neural and glial cells, inhibited the inflammatory response. Clinical application of MSCs-based therapy has been developed due to its accessibility and low immunogenicity (18,19). A recent clinical study reported that, in their phase I study, intraventricular transplantation of allogeneic human UCB-derived MSCs into severe intraventricular haemorrhage (IVH) preterm infants was safe and feasible. Nine premature infants received cell transplants at 11.6±0.9 postnatal days, three received low-dose injections (5×10^6 cells/kg) and six received high-dose injections (1×10^7 cells/kg), no serious side effects and dose-limiting toxicities were observed. Cerebrospinal fluid (CSF) biomarkers like vascular endothelial growth factor (VEGF), and brain-derived neurotropic factor (BDNF) exhibited increase in some infants after MSCs intervention compared with baseline values (18). These data support the neuroprotective activity of transplanted MSCs in the treatment of WMI.

Oligodendrocyte precursor cells (OPCs) transplant

OPCs are widespread in central nervous system, most of them differentiate into oligodendrocytes and a few into astrocytes. Oligodendrocytes loss or dysfunction will cause demyelination, which is the main pathological feature of WMI. Myelin regeneration is mediated by OPCs, thus they are considered as seed cells for treating demyelinating diseases including WMI. OPCs can be obtained from fetal brain tissue or derived from ESCs/iPSCs, NSCs, or transdifferentiated from somatic cells like fibroblast (20-22). In early stem cell studies, mouse primary OPCs were isolated and transplanted into WMI mouse/rat brain. Experimental data suggested that rat primary OPCs can survive and migrate in the host brain and promote the secretion of neurotrophic factor (23). Proliferated NSCs ($BrdU^+/Nestin^+$) increased in treated animals and these animals showed relived behavior deficits compared to sham operated controls (24). These studies suggest that the transplantation of OPCs have neuroprotective effects and can promote endogenous nerve regeneration. More important, it served as a source for myelin repair has been repeatedly reported. Porambo *et al.* (25) reported that intra-callosal injection of glia progenitor cells derived from embryonic spinal cord 17 days after HI in PND5 mice was associated with increased MBP density in cell treated WMI mice despite limited cell survival. Human ESCs derived OPCs were not applied in that time because of oligodendrocytes differentiation from human ESCs were not possible in the past. Transplantation of human primary NSCs derived OPCs seems like more feasible (26). Wu *et al.* (26) transplanted OPCs which were isolated from human aborted embryo into the forebrain of HI rats. They found that the myelinated axons were increased significantly in lesion site 90 days after transplantation. These results also showed long term survival of transplanted human OPCs in WMI rats. As the technical difficulties of human ESCs differentiation have been overcome, preclinical studies on human oligodendrocytes transplantation have

also been carried out. Kim and his colleagues showed that neurobehavioral performance were improved when human NSCs derived OPCs being transplanted for 3 days in HI animal models. Transplanted cells migrated to the injury site, differentiated into mature oligodendrocytes, expressing MBP and wrapped the neuronal cells to form new myelin sheaths (27).

Discussion

WMI is primarily caused by perinatal hypoxia and ischemia. Full-term infants and premature infants have different patterns of injuries when exposed to HI owing to late oligodendrocyte progenitors' selectively vulnerable. Preterm infants are characterized by periventricular leukomalacia while the gray matter is predominantly injured in full-term infants (9). At present, the therapeutic methods of WMI in premature infants are mainly symptomatic support and mild hypothermia, while the effect of these treatments is limited in some severe cases. Cell transplantation is a promising treatment for these cases. The therapeutic mechanisms of cell transplant include replacing the lost cells, secreting neurotrophic factors, modulating inflammatory process, promoting endogenous neurogenesis, stimulating angiogenesis, and so on. However, the determined mechanisms are not fully revealed. Understanding the mechanism of stem cell therapy will be much more conducive to the future utilization. MSCs transplantation for WMI has been demonstrated to be safe in clinical trials (18). Gene editing in combination with stem cell therapy is also under pre-clinical researches (15). Although OPCs can specifically supplement the lost oligodendrocytes, its clinical application has not yet been developed due to the difficulty of obtaining OPCs. With the development of cell differentiation technology, OPC differentiated from ESCs/iPSCs will be wildly used in the treatment of WMI.

Conclusions

Preclinical studies have confirmed the safety and feasibility of different cell types for transplantation. NSCs and MSCs transplantation therapy has entered clinical trial processes, whereas OPCs transplant has not been used in clinical studies yet owing to its differentiation difficulties (18). In addition, more experimental data is needed so that we can choose the optimal cell type, transplant dose and transplant site to enhance the treatment effects and avoid immune rejection.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://pm.amegroups.com/article/view/10.21037/pm-20-49/coif). WZ serves as an Executive Editor-in-Chief of *Pediatric Medicine*. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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