Mesenchymal Stem Cell Therapy for Hearing Loss: Review of Literature

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Abstract

Hearing loss is a worldwide problem. Sensorineural hearing loss (SNHL) is the most common sensory deficit. In mammals, hair cells and auditory neural cells are terminal cells that can regenerate after damage, rendering SNHL a permanent problem. The current solutions for SNHL include hearing aids, cochlear implants and hearing assisting devices. Stem cell therapy provides hope for replacing damaged hair cells and auditory neural cells. There are three basic types of stem cells: embryonic stem cells, induced pluripotent adult stem cells, and mesenchymal stem cells. Mesenchymal stem cells represent an interesting choice in stem cell therapy. There are many parameters that affect the outcome of stem cell therapy for hearing loss. The in-vivo animal research regarding stem cell therapy for hearing loss is heterogeneous regarding those parameters. This article explores stem cell therapy in hearing loss in general, focusing on mesenchymal stem cells, which are the most used stem cell type.

Key words

Stem cell therapy; mesenchymal stem cell; hearing loss; sensorineural hearing loss

Introduction

Sensorineural hearing loss (SNHL) is the most prevalent type of hearing loss, arising from damage to the inner ear or the auditory nerve. In adult mammalian cochlea, hair cells and spiral ganglion neurons are terminal cells and do not regenerate after injury. Stem cell-based therapy provides a potential solution for hair cells and auditory neurons regeneration and/or replacement. The research in stem cell-based therapy is of two kinds: activation of endogenous stem cells/progenitor cells, and transplantation of exogenous stem cells. There are three types of exogenous stem cells: embryonic stem cells, induced adult pluripotent stem cells, and mesenchymal stem cells [(1)].

Mesenchymal stem cells (MSCs) are the most tested and used cells for cell-based therapy. They were first described 30 years ago, with over more than 55,000 publications available today. About 1000 clinical trials are registered for MSCs with ten studies in phase 4, showing promising results, especially for treating osteoarthritis and heart ischemia. MSCs have shown remarkable tissue repair and regenerative properties. MSCs were first identified as a subpopulation of bone marrow cells which are plastic adherent, have the ability to differentiate into osteogenic, chondrogenic, and adipogenic fates, express CD105, CD73, and CD90, and lack haemopoietic markers. Further studies were able to demonstrate the ability of MSCs to differentiate to other cell fates . In vitro studies demonstrated the ability of both animal and human bone marrow derived MSCs to differentiate into hair cell-like cells and auditory neuron-like cells. Hair cell-like hair cells have also been obtained from MSCs from other sources like adipose tissue [(2)].

Heterogeneity of in vivo research

The *in-vivo* animal studies of stem cell therapy for hearing loss are still in their early phases. According to a recent review and careful search in PubMed, we identified a total of 24 animal studies that evaluated in-vivo stem cell therapy with the aim to treat inner ear diseases [(3)].

There are several variables in the research of stem cell therapy to consider. Of these are the disease model, the animal species, the anthropometric characteristics of the animal, the source of stem cells, the degree of differentiation of stem cells prior to transplantation, number of passages of cultured stem cells, the route of delivery, timing of stem cell transplantation since inner ear injury, dose of stem cells, and number of transplantations. Each of the aforementioned factors need to be systemically examined to elucidate their relative contribution to the final outcome of stem cell therapy. Giving the multitude of factors in the study of stem cell therapy and the paucity of the in-vivo studies, the current studies are widely heterogenous. Moreover, the studies varied in terms of outcome measures.

To highlight the heterogeneity of the current studies, we can observe that there are no two studies that match on the following three parameters: source of stem cells, disease model, and route of delivery of stem cells.

On one hand, the heterogeneity of research could impose difficulties in comparison between reports. On the other hand, most of the research reported positive outcomes

with stem cell therapy, despite the wide variability between the studies, which indicates a beneficial redundancy of stem cell therapy and a promising modality of treatment.

Source of stem cells

Of the 24 in-vivo animal studies, there were 19 studies which used mesenchymal stem cells for treatment of inner ear diseases. There were 8 studies which evaluated MSCs derived from bone marrow for treating SNHL and auditory neuropathy in animal models. In the remaining 11 studies, mesenchymal stem cells were isolated from adipose tissue (n=3), human umbilical cord (n=4), human placenta (n=1), skin (n=1), harderian gland (n=1), and human limbus (n=1). Other types of (non-mesenchymal) stem cells in the in-vivo studies included olfactory epithelium (n=2), tongue epithelium (n=1), and human embryonic stem cells (n=2) [3] .

Mesenchymal stem cells represent an interesting choice for cell-based therapy due to the diversity of sources, simplicity of isolation, immunomodulation, anti-inflammatory effect, ability to migrate and home to injured tissues, minimal teratogenicity, and limited ethical issues that are associated with embryonic stem cells.

Disease model

Studies used different injurious agents to induce SNHL. There are two other studies used platinum-based compounds (cisplatin and carboplatin) to induce SNHL and evaluated MSC transplantation. One study evaluated skin derived MSCs intravenous transplantation to treat cisplatin induced SNHL in a mouse model [4]. The other study evaluated intravenous transplantation of harderian gland derived mesenchymal stem cells to treat carboplatin induced SNHL in guinea pig model [5]. Tsia used two protocols for disease induction with IP cisplatin (4 m g/kg/day for 5 days, and 25 mg/kg once injection) [4]. Abdel El Raouf et al used two doses 24 mg/kg intraperitoneal injection of carboplatin 2 days apart [5]. Previous studies showed that cisplatin through different protocols of injections results in elevated hearing thresholds [6].

Two studies used other ototoxic drugs: kanamycin and neomycin (both used BM-MSC) [7, 8]. Four studies used Ouabain to selectively injure spiral ganglion cell to obtain auditory neuropathy models (2 studies used BM-MSC) [9-12]. Two studies used a combination of Ouabain and neomycin [13, 14]. Two studies used 3-nitropropionic acid (3NP) to selectively inure Stria Vascularis and spiral ligament (both used BM-MCS) [15, 16]. Two studies used β tubulin to induce autoimmune inner ear disease [17, 18].

Eight studies used acoustic trauma to achieve a model of noise induced SNHL (2 studies used BM-MSC) [19-26]. Two studies used a congenitally deaf animal model [27, 28].

Not only the disease induction agent was different between studies, but also each study implemented different protocol for disease induction with the same agent. The fact that most studies reported positive results with stem cell therapy in different disease models, highlights the ability of the stem cell therapy to treat a variety of inner ear lesions.

Timing, Dose and Frequency of stem cell injection

Timing

All studies administered stem cells early after injury. The time window from disease induction to stem cells administration ranged from 1 day [7] to 4 weeks [9]. The microenvironment in damaged cochleae in early injury may have been favorable to invite the transplanted stem cells into the site of injury through chemical mediators released from injured tissues. A study noticed that stem cells home in injured cochlea but not in uninjured cochlea [21]. Moreover, the early injury can be more easily reversible through stem cell treatment than a longstanding damage with more irreversible pathology. Most patients with SNHL have chronic type of pathology rather than acute insult. Accordingly, more in-vivo studies are needed to evaluate the efficacy of stem cell therapy to treat a longstanding damage in the cochlea.

Dose

The dose of stem cell therapy was variable. Systemic delivery needed higher doses that local delivery of stem cells. In systemic delivery, stem cell dose ranged from 5×10^5 [23] to 1×10^7 [13] cells per injection among different studies. In local delivery, the dose ranged from 1×10^5 [16] to 1×10^6 [22] cells per injection among studies. More studies are required to elucidate the optimum dose for each approach and disease model.

Frequency

Most studies injected one dose of stem cells per animal [3]. Only two studies injected stem cells intraperitoneally weekly for 6 weeks in β tubulin induced inner ear autoimmune disease [17, 18]. Of note, a study evaluated the survival of stem cells 3 months after transplantation and did not find any surviving cells [11], raising the question if more doses are needed in stem cell therapy.

More studies are needed to evaluate the effect of one versus multiple doses of stem cell therapy and the optimum interval between doses in different disease models.

Status of stem cells

Studies have showed that increased number of passaging is associated with MSC senescence and decreased proliferation potential [29]. A study demonstrated that MSC senescence occurred past passage 6 . Not all studies reported passaging status. In the studies reporting passaging status, the number of passages ranged from 3 [21] to 15 passages [16]. More studies are needed to evaluate the effect of number of passages on the outcome of MSC transplantation.

Most of the studies (22/24) and ours transplanted native stem cells without any in-vitro induction/pre-differentiation. Two studies transplanted neural induced MSC [8, 12] . The therapeutic benefit of native vs induced MSC transplantation need further comparative studies.

Route of delivery

The route of stem cell delivery can be divided into local and systemic routes. The majority of local administrations aimed at injecting transplanted stem cells into the perilymphatic space. The perilymph fluid is low in potassium which make it less hostile and detrimental to the transplanted stem cells than the endolymph fluid [30]. From the site of injection, the transplanted cells can migrate to the site of injury inside the cochlea.

In previous in-vivo studies, the perilymphatic space was accessed through the round window (n=2, one study injected BMSC) [20, 24], lateral wall cochleostomy of basal turn (n=7, two studies injected BMSC) [8, 9, 12, 19, 22, 25, 27], and semicircular canals approaches (n=2, both studies injected BMSC) [15, 16].

Round window approach as it is a natural gate to Scala tympani with minimal trauma. Moreover, the round window approach can be a less invasive approach that can be accessed easily in humans.

Other local delivery approaches include: injection into the modiolus (n=2) [9, 10], into the subarachnoid space (n=2) [26, 28], and around cochlear nerve fibers (n=1) [11].

The systemic delivery of stem cells can be achieved through intravenous (n=7) [4, 5, 7, 13, 14, 21, 23] and intraperitoneal (n=2) [17, 18] approaches. Although these approaches are attractive as they are less invasive and can be easily performed, there are some considerations to be taken in account. One study demonstrated trapping of most transplanted cells in the lung, which limits the available stem cells that reach the organ of interest [21]. Moreover, systemic administration of stem cells carries the risk of

tumorigenesis in different parts of the body, although studies showed that the risk is more with embryonic and induced stem cells than with mesenchymal stem cells [30].

More studies are needed to explore the best route to deliver MSCs to the cochlea in different disease models.

Migration and Homing

Several studies demonstrated the ability of MSCs to migrate from initial site of transplantation to different target sites. There are different methods to study stem cell migration and homing.

Previous studies showed that transplanted stem cells implant themselves favorably at sites of injury rather than normal tissue [7, 21]. As cisplatin inflicts injury in stria vascularis, organ of Corti, and spiral ganglion, this widespread injury may have contributed to attract the transplanted cells to the different sites of injury in our study.

The ability of BM-MCS to migrate from initial site of injection to different locations inside the cochlea was demonstrated by other studies.

Some studies transplanted human BMSC (HBMSC) in animal models and looked for human DNA and antigens [7, 8, 12, 21]. Human BM-MSC were evident in cochlear histological samples by looking for human DNA by PCR [7] and immunohistochemistry staining of HLA ABC [7], and human-specific nuclear antigen [8, 12, 21]. Choi B.Y. et al reported homing of HBM-MSC only in spiral ganglion in noise exposed rats, but not in other locations inside the cochlea after systemic infusion [21]. Bettini et al reported homing of HBM-MSC in modiolus, lateral wall, around small capillaries, but not in organ of Corti or spiral ganglion, after intravenous transplantation in kanamycin deafened mice [7]. Jang et al reported HBM-MSC migration from scala tympani (initial site of injection) to multiple sites inside the cochlea including stria vascularis, spiral ligament, organ of Corti, spiral limbus, spiral ganglion and around cochlear nerve fibers in neomycin deafened guinea pigs [8]. Cho et al reported HBM-MSC migration from scala tympani (initial site of injection) to only spiral ganglion in Ouabain deafened guinea pigs [12].

Two studies used green fluorescent protein (GFP) positive BM-MSC harvested from transgenic animals and was able to demonstrate stem cell homing by immunohistochemistry staining for GFP [9, 15]. Kada et al injected GFP positive BM-MSC in posterior semicircular canal in 3NP treated mice. Kada et al reported that most GFP-expressing BM-MSCs was found the perilympahtic space and adherent to the

structures in the perilymphatic space. Few cells were found inside cochlear tissue including spiral ligament, spiral limbus and spiral ganglion [15]. Matsuoka et al reported finding GFP positive BMSC in every turn in the cochlea, in scala tympani, scala vestibuli, and modiolus in normal and Ouabain treated gerbils, after modiolar and perilymphatic administration of BM-MSC [9].

Kamiya et al injected Bromodeoxyuridine (Brdu) labelled BM-MSC into scala tympani through the lateral semicircular canal. They observed positive fluorescent cells at the ampullary crest, scala vestibuli, lateral wall of scala media, and spiral limbus. Moreover, tissue invasion of transplanted cells was observed with higher rates in injured versus uninjured cochleae [16].

Other studies demonstrated the ability of stem cells from other sources to migrate inside the cochlea into different locations.

Two studies used GFP positive stem cells in their study of noise induced SNHL animal models [20, 22]. A week after transplantation through round window, Fetoni et al found that GFP positive adipose derived MSC in perilymphatic space, scala tympani, scala vestibuli, strai vascularis, Reissner's membrane, and basilar membrane in close proximity to Rosenthal canal [20]. Xu Y.P. et al found GFP positive neural-induced olfactory epithelium stem cells in endocochlear fluid and some cells migrated to spiral ganglion, after transplantation through cochleostomy [22].

Some studies used human derived stem cells and looked for human DNA or antigens in transplanted cells [11, 18, 23]. Yoo et al administered human adipose derived MSC intraperitoneally in β tubulin induced autoimmune inner ear disease in mice. Yoo et al demonstrated stem cells homing in the stria vascularis and in the spiral ligament by immunostaining [18]. Ma et al injected human umbilical cord MSC into the subarachanoid space of congenitally deaf albino pig. Ma et al identified human Perinuclear centromere associated protein (PNCA) positive cells in cochlea in spiral ligament, organ of corti, stria vascularis, and spiral ganglion. Chen H.C. et al injected human limbus derived MSC around auditory nerve fibers via suboccipital approach in Ouabain induced auditory neuropathy mice model. They found stem cells in the modiolus by anti-human nuclear antibody staining [11]. Kim et al injected human embryonic stem cells derived MSC intravenously in noise induced SNHL in rat model. They looked for human Syr gene by PCR and STEM121 (cytoplasmic protein) by

immune-staining to localize transplanted cell. They found transplanted cells in spiral ganglion but no cells in outer or inner hair cell regions [23].

Two studies used Vybrant CM-DiI (a lipophilic dye) labelled stem cells [19, 27]. Pandit et al injected labelled olfactory epithelium stem cells into congenitally deaf mice through cochleostomy. They found stem cells in scala tympani and vestibuli, but without integration into cochlear tissue [27]. Sullivan et al injected labelled tongue epithelium stem cells into noise-induced SNHL in mice through cochleostomy. They found stem cells in spiral ligament, scala vestibuli, Reissner's membrane, and the squamous epithelial lining of scala tympani 1 week after transplantation [19].

Abd El Raouf et al injected harderian gland derived MSC harvested from male guinea pigs into carboplatin deafened female guinea pigs intravenously. They detected Sry gene via real time PCR in cochleae of stem cell treated guinea pigs but not in controls [5].

Xu L. et al injected super magnetic iron oxide (SPOI) labelled human umbilical cord derived MSC in the subarachnoid space of noise induced SNHL pig model. They identified stem cells using Prussian blue stains, scanning electron microscopy x-ray dispersive analysis, and transmission electron microscope. Stem cells were found in scala tympani and spiral ganglion cells but not in outer or inner hair cell regions [26].

The aforementioned reports demonstrate the ability of MSC to migrate from initial site of transplantation and their homing into different locations inside the cochlea.

The Role of Microenvironment

Studies showed that the microenvironment influence both homing and differentiation of implanted stem cells. Mesenchymal stem cells were found to get implanted in sites of injury in damaged cochlea but does not implant in undamaged cochlea [7, 21]. Furthermore, the site of implantation of transplanted stem cells influences their fate of differentiation. A study showed that mesenchymal stem cells implanted into organ of Corti expressed hair cell markers [31]. These studies demonstrate the importance of cochlear microenvironment in homing and differentiation of transplanted stem cells.

An interesting observation came from studies that systemically delivered stem cells. Two studies injected BM-MSC in normal control groups alongside diseased groups [7, 21]. Choi B.Y. et al detected transplanted BM-MSC in noise and neomycin injured cochleae, but not in intact cochleae without injury [21]. Bettini et al identified BM-MSC only in cochlea pretreated with kanamycin. In these studies, the systemically

administered BM-MSC only got implanted in injured cochlea [7]. This demonstrates the importance of the microenvironment created by injury to attract transplanted stem cells to implant in the site of damage.

Another interesting observation comes from studies that inflict selective injury in the cochlea and deliver stem cells locally. Kamiya et al applied 3NP to round window in rats. This caused selective damage to fibrocytes in cochlear lateral wall and to a lesser degree in spiral limbus. Organ of Corti and spiral ganglion were spared of injury. The transplanted BM-MSC through the lateral semicircular canal got implanted in the lateral wall and to a lesser degree in the spiral limbus, but not in organ of Corti or spiral ganglion. The transplanted stem cells assumed the morphology of cochlear fibrocytes and expressed gap junction proteins (connexin 26 and 30) [16]. Cho et al found that transplanted HBM-MSC migrated to Ouabain injured spiral ganglion after injection through cochleostomy, and expressed NF-H (neural marker) suggesting neural differentiation [12]. Similarly, Chen H.C. et al found that human limbus derived MSC localized into modiolus 2 days after stem cell injection around cochlear nerve, in Ouabain injured mice [11]. These studies demonstrated that MSC selectively implant themselves in sites of injury, highlighting the importance of microenvironment for stem attraction and differentiation.

Survival of MSC After Transplantation

Little is known about long-term survival of MSC inside cochlea after injection. The maximum period between BM-MSC transplantation and stem cell identification in cochlea ranged from one week [9] to 8 weeks [8] among in-vivo studies aimed at evaluating BM-MSC in treating cochlear diseases. Whereas, among all in-vivo studies aimed at evaluating stem cells from different sources in treating cochlear diseases, the maximum period between stem cell transplantation and stem cell identification in cochlea ranged from 5 days [19] to 8 weeks [8]. Jang et al reported survival of human derived BM-MSC in neomycin deafened cochleae of guinea pigs 8 weeks after transplantation through cochleostomy, which is the longest reported period of survival [8]. On the other hand, Chen H.C. et al reported negative immunostaining of human limbus derived MSC in mice cochleae pretreated with Ouabain 3 months after transplantation, despite initial positivity 2 days post transplantation. Despite negativity at 3 months, they reported improvement of spiral ganglion cell counts compared to control [11]. Xu Y.P. et al examined olfactory stem cell survival 3- and 5-days post

transplantation through cochleostomy in rats with noise induced SNHL. They reported decreasing numbers of stem cells in day 5 compared to day 3, indicating early loss of stem cells [22]. Whether early loss of stem cells post transplantation occur with other types of stem cells needs to be studied.

As the in-vivo studies are heterogenous and there are no enough long-term survival studies, the factors affecting survival of stem cells post transplantation in cochlea need further studies to be elucidated.

Outcome of BM-MSC Transplantation

The majority of the in-vivo studies reported improved histopathological and/or functional outcome after transplanting mesenchymal stem cells in different animal models.

Two studies used platinum-based compounds (cisplatin and carboplatin respectively) to induced SNHL in animals [4, 5]. Tsai et al reported decreased ABR thresholds in mice treated with skin derived MSC. Immunohistochemistry and gene expression studies showed down regulation of genes associated with apoptosis [4]. Abd El Raouf et al reported significantly increased counts of hair cells and spiral ganglion neurons in BM-MSC treated group. Scanning electron microscope showed normal arrangement of hair cells in BM-MSC treated group [5].

Mesenchymal stem cell treatment was beneficial in other inner ear disease models. Auditory neuropathy animal model was achieved in two studies by using Ouabain [11, 12]. Both studies reported improved ABR thresholds and spiral ganglion histological examination in MSC treated groups. Cho et al reported localization of MSC in spiral ganglion, the site of injury, by immunohistochemistry [12].

Some studies evaluated MSC treatment for noise induced SNHL [7, 16, 26]. Although Kamiya et al reported no significant improvement of hearing threshold after MSC treatment, both Bettini et al and Xu L. et al reported significant improvement of hearing threshold. Kamiya et al reported increased expression of trophic factors associated with survival by immunohistochemistry [16]. Bettini et al reported upregulation of genes related to hypoxia response, mitochondrial dysfunction, immunomodulation and regulation of apoptosis [7].

Yoo et al and Zhou et al evaluated MSC in Autoimmune inner ear disease animal model induced by β-tubulin. Both reported improved hearing thresholds in MSC treated groups, and downregulation of the autoimmune response [17, 18].

Choi et al and Kil et al used both neomycin and Ouabain to induce injury in hair cells and spiral ganglion. They both reported improvement of hearing thresholds and histological examination of MSC treated groups [13, 14].

Ma et al evaluated MSC in congenitally deaf albino pigs. Ma et al reported improved hearing thresholds [28].

Mesenchymal stem cells show great potential to treat a variety of inner ear diseases.

Mechanisms of BM-MSCs in Cochlear Healing and Regeneration

The beneficial therapeutic effect of BM-MSCs transplantation can be explained by a number of mechanisms which include direct trans-differentiation, induction of inner ear progenitor cells, paracrine effect, secretion of exosomes, mitochondrial transfer, and cell fusion.

Trans-differentiation

BM-MSCs can directly trans-differentiate and replace damaged cells. The ability of BM-MSCs to differentiate to many cell types has been demonstrated by several studies in-vitro. BM-MSCs can differentiate into fibroblast-like cells, hair cell-like cells, and auditory neuronal cell-like cells [30]. In-vivo, the molecular cues in the microenvironment can help determine the cell fate of the transplanted BM-MSCs .

During cochlear development, the epithelium lining of the early cochlear duct is divided into five distinctive regions with unique molecular expression patterns, which commit each region to certain cell fates. Moreover, cell-to-cell contact through molecular signals influence cellular differentiation [32]. This unique molecular expression patterns by different regions in the cochlea may provide cues to the transplanted MSC to trans-differentiate into certain fates. In-vivo studies demonstrated this concept. A study demonstrated MSC implantation at cochlear lateral wall led to differentiation into cochlear fibrocytes after selective injury to the stria vascularis by 3NP [16]. Another study demonstrated that transplanted MSC that got implanted in organ of Corti expressed hair cell markers [31]. In auditory neuropathy model, where spiral ganglion was selectively injured by ouabain, the transplanted mesenchymal stem cell implanted in spiral ganglion and expressed neuronal markers [12].

Further studies are needed to investigate MSC trans-differentiation as a possible mechanism for inner ear regeneration in different disease models.

Induction of Inner Ear Progenitor Cells

Recent research has identified evidence of the ability of Lrg5+ supporting cells to proliferate and differentiate to sensory epithelium. The candidate inner ear progenitors are inner border cells, inner pillar cell, and third row Dieter's cell [33]. Transplanted BM-MSC, through the release of trophic factors and molecular signals, may have induced the inner ear progenitor cells to differentiate and replace degenerated cells.

Further studies are needed to explore the possible role of MSC transplantation to induce inner ear progenitors to replace damaged sensory epithelium.

Paracrine Effect

The paracrine activity of transplanted BM-MSC may have a role in promoting cochlear healing and regeneration. Currently, a number of studies have shown that in-vivo MSC transplantation can promote tissue repair by secreting paracrine factors. This beneficial effect was demonstrated in different disease models including myocardial infarction, cerebral ischemia, limb ischemia, burn injury, acute kidney injury, and liver injury. MSCs secrete a number of factors that include a variety of growth factors, cytokines, and microRNAs. These paracrine factors can exert anti-apoptotic, anti-fibrotic, neuroprotective, pro-proliferative, and pro-angiogenic functions.

Further studies are needed to unravel the role of paracrine factors secreted from transplanted MSC in cochlear regeneration.

Extracellular Vesicles

Transplanted BM-MSCs secrete exosomes which are extracellular vesicles with size ranges from 30–120 nm. The most important capabilities of these vesicles are to transport essential macromolecules like proteins, lipids, cell surface receptors, enzymes, cytokines, transcription factors, mitochondria and genetic material to be endocytosed by neighboring injured cells. MSC-derived exosomes have been investigated in brain injury, cardiovascular disease, graft vs. host disease, skin wound healing, and COVID-19 among others . MSCs derived exosomes may have a role in cochlear regeneration after injury. This role needs more studies to be further clarified.

Mitochondrial Transfer

Mitochondrial transfer is another mechanism by which the transplanted BM-MSC can salvage cisplatin-injured cells in stria vascularis, organ of Corti, and spiral ganglion.

Mitochondria are responsible for generating energy through oxidative phosphorylation, involved in cytosolic calcium regulation, different biochemical reactions and regulation of apoptosis. When mitochondria become dysfunctional, they produce less energy and

increase the production of reactive oxygen species (ROS), which can damage DNA, proteins, and lipids [2].

Studies have shown that MSCs transfer mitochondria to injured cells to improve cellular function and promote tissue regeneration in various disease models. Mitochondrial transfer was observed to occur through nano-tunnels between cells, gap junctions, cell fusion, secretions of micro-vesicles containing mitochondria, and direct uptake of isolated mitochondria. The transferred mitochondria integrate into the recipient cell mitochondrial network, enhancing their bioenergetics and reducing oxidative stress [2]. The beneficial effect of mitochondrial transfer was demonstrated in different disease models. Boukelmoune et al. proposed that MSCs can transfer mitochondria to neural stem cells (NSC) to protect against neurotoxic effects of cisplatin treatment [34]. In cardiac ischemia, MSC mitochondrial transfer has been shown to improve cardiac function and reduce infarct size. In lung diseases, MSC mitochondrial transfer has been shown to reduce inflammation and improve airway function. In neurodegenerative diseases like Alzheimer's and Parkinson's, MSC mitochondrial transfer has been shown to protect neurons from death and improve cognitive function [2].

Further studies are needed to investigate the role of mitochondrial transfer by transplanted MSCs in cochlear healing and regeneration.

Cell Fusion

Cell fusion, the process by which two or more cells merge to form a single hybrid cell, has been recognized as a critical mechanism contributing to MSC-mediated healing. Fusion events between MSCs and host cells can lead to the transfer of genetic material, mitochondria, and other cytoplasmic components, thereby enhancing the regenerative capacity of the recipient cells. Huda and colleagues demonstrated the ability of injected fetal MSCs to rescue neurons in the cerebella of symptomatic aged mice, which selectively fuse with injured Purkinje cells and interneurons but, interestingly, not with healthy neurons [35]. Further studies are needed to investigate cell fusion as a mechanism by which transplanted MSCs can promote cochlear healing and regeneration.

Safety of Transplantation

Mesenchymal stem cells are hypoimmunogenic. They lack the expression of MHC II. They downregulate inflammation and modulate immune response. These characteristics make allogenic and even xenogenic MSC treatment possible.

Kasagi et al transplanted allogenic BM-MSCs in normal young and adult mice groups through post semicircular canal approach. Kasagi et al reported no adverse effect of the transplanted BM-MSC on hearing thresholds [36]. This study indicates that BM-MSCs does not interfere adversely with normal cochlear function.

Two studies showed that trans-tympanic injection of BM-MSCs were not associated with any inflammatory response or adverse effects on rat cochlear function [37, 38].

Bettini et al systemically injected human BM-MSCs and human adipose MSC in kanamycin treated mice. Bettini et al reported detection of MSCs in liver, kidney and spleen with no pathological adversaries after 30 days of transplantation [7].

Studies which transplanted allogenic MSC in their animal models did not report any signs of rejection [4, 5, 16, 20]. Moreover, many studies transplanted human MSCs in animal models (xenogenic transplantation) and did not report any signs of rejection [11, 13, 14, 18, 21, 25, 26, 28].

Human Studies

Although there are several animal studies which demonstrated the potential of MSC to treat SNHL in animal models, there is only one case report about BM-MSCs transplantation in two SNHL patients. In this report, autologous BM-MSCs were transplanted intravenously in two patients with longstanding SNHL. There were no reported complications reported by the patients. Hematological, biochemical and coagulation profile 12 months and 3 years after transplantation were normal. However, there was no improvement in auditory threshold [39].

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