REVIEW ARTICLE

Brainstem Response Changes of Noise-Induced Auditory Damage after Stem Cell Injection: Systematic Review of Animal Studies and Meta-Analysis

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ABSTRACT

Objectives: As permanent hearing loss is irreversible, individuals often rely on hearing aids or cochlear implants as effective rehabilitation solutions. Stem cell therapy has been used this disability at the level of preclinical studies and effectiveness has not been determined to improve of auditory brainstem response (ABR) and restore damaged hair cells of cochlea. This systematic review shows the role of stem cell injection on ABR threshold changes in noise induced hearing loss (NIHL) models.

Materials & Methods: Search strategies for Embase, PubMed, and Web of Science were performed based on keywords stem cell, hearing loss, and noise from 2000 to 2024. Nine studies were evaluated for study variables and risk of bias, and only six studies were analyzed to estimate effect size of auditory threshold.

Results: The broadband noise was the most common noise to create NIHL model. The improvement of ABR threshold was estimated -2.46, 95% (CI: -3.26, -1.66) after stem cell injection. Significant mean differences were observed in ABR-threshold for local injection (n = 148) and mesenchymal stem cell (MSC) injection (n = 129) with high heterogeneity between studies.

Conclusion: According to high heterogeneity between studies, it may be concluded that MSC, systemic injection, and human-derived cells are effective in recovery of auditory brainstem function.

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Introduction

The most common of hearing loss is sensorineural hearing loss (SNHL), involving damage to the hair cells or auditory nerve. This disorder has multiple causes, such as noise over exposure, the use of ototoxic drugs, and genetic mutations in the cochlea or auditory nerve pathways (1). Unfortunately, most cases of this disorder are irreversible and induce permanent hearing damage (1, 2). Currently, no clinical therapies are available to repair hearing function, and only rehabilitation tools like hearing aids and cochlear

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implantation with hearing training have effective roles in promoting hearing function (3-5).

Over the last few decades, the discovery of cochlear hair cell regeneration in avians has spurred extensive research into stem cell-based therapies for SNHL in animal models (6). Research has been performed on cell injection into the inner ear using different types of stem cells through varied access routes, such as round window, lateral wall of cochlea and intravenous injection (6-9). Its efficiency has been usually evaluated by auditory brainstem response (ABR) threshold and morphological assessment (10). The success rate of cell transplantation in the cochlea, as in other organs, depends on the type and source of stem cells prepared, the timing of injection, and the dose of cells (11). Given the environment of the cochlea and its complex structure, cell injection also depends on the degree and mechanism of damage and the route of cell injection (3). Some studies did not find the function integration of injected cells in the auditory system (9, 12), whereas other studies reported hearing restoration in animal models with ototoxic drugs that had local injection of exogenous stem cells, particularly mesenchymal stem cells (MSCs), and found improvement in ABR threshold (6, 13). A review article reported the efficacy of MSCs in repairing hearing damage, specifically caused by ototoxic drugs, despite the heterogeneity of the studies (14). This study has less investigated studies with animal models of noise-induced hearing loss (NIHL).

NIHL is a type of acquired SNHL that the World Health Organization estimates its prevalence to be almost one-third of all hearing loss worldwide (15). Differences in the mechanisms of cochlear damage exist in NIHL compared to ototoxic drug-induced hearing loss. NIHL occurs with direct mechanical destruction like damage to stereociliary structure, tear of the organ of Corti from the basilar membrane, disarranging of cell junctions, and mixing of endolymph and perilymph, as well as metabolic destruction, such as cell death with generation of reactive oxygen species, rising of free calcium in outer hair cells, and excessive glutamate release (16). Animal studies investigating cell-based therapies for NIHL exhibit significant variability, particularly regarding the specific stem cell types utilized, the routes of administration, and the subsequent auditory system responses (8, 17-19). All of them have not been pooled and analyzed, and it is questionable role of cell injection in NIHL on ABR threshold. Accordingly, this study aims to investigate the changes in ABR threshold in animal models of NIHL. Its results can certainly be useful to guide future studies in experimental fields and clinical trials to improve auditory function in hearing-impaired children.

Materials & Methods

Search strategies

This study is reported based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines (20). The search strategy was guided by a clearly defined study aim and the PICO framework. The PICO was defined as follows: Patient (P): animal model with NIHL, Intervention (I): stem cell injection, Comparison/Control (C): NIHL group without cell injection, Outcome (O): change of auditory threshold in ABR. Compressive searches were performed from Embase, PubMed, Web of Science, Turning Research Into Practice, and ProQuest from 2000 to 2024. Search strategies were based on keyword combinations, including “stem cell”, “noise”, “hearing loss” and “acoustic trauma”. The searches were first screened based on title/abstract and then reviewed full-text in English. The screening was independently done by two investigators. Two other investigators helped to achieve agreement to resolve any difference of opinions. In the final search, reference lists of recorded articles were manually reviewed to find additional sources. All records retrieved from the databases were imported into an EndNote library. Upon completion of the search process, duplicates were identified and removed.

Inclusion and exclusion criteria

The inclusion criteria were full-text original papers in English language and experimental studies (in vivo models) with randomized or not. Those studies have investigated the therapeutic effects of stem cells on NIHL model regardless of methodological quality.

The exclusion criteria were clinical trials of cell transplantation, in vitro models, review articles and editorial letters, experimental studies without control group, and SNHL models due to ototoxicity and hereditary. Treatment with exosomes, microRNA, micro vesicles, and other biological products was excluded in this review.

Primary and secondary endpoints

The main outcome of this systematic review was the assessment of hearing function using ABR. Restoration of sensory epithelium in the organ of Corti was considered a secondary outcome.

Data extraction

The animal groups included a control group and an experimental group in which the second received stem cells (mesenchymal stem cell, mononuclear cell, neural stem cell, and the like). Two researchers collected data with the monitoring of two senior investigators. The extracted data consisted of the characteristics of studied

animals (species, age, gender, and weight), number of animals in each group, condition of noise exposure (frequency, intensity, and duration), profile of transplanted stem cells (source, origin, cellular density, and route of injection), time assessment after injection, ABR threshold, and the integrity of the organ of Corti structure. The quantitative data was collected from tables. Data of graphs or plots were determined using software of plot digitizer online. (Available from: https://plotdigitizer.com/app).

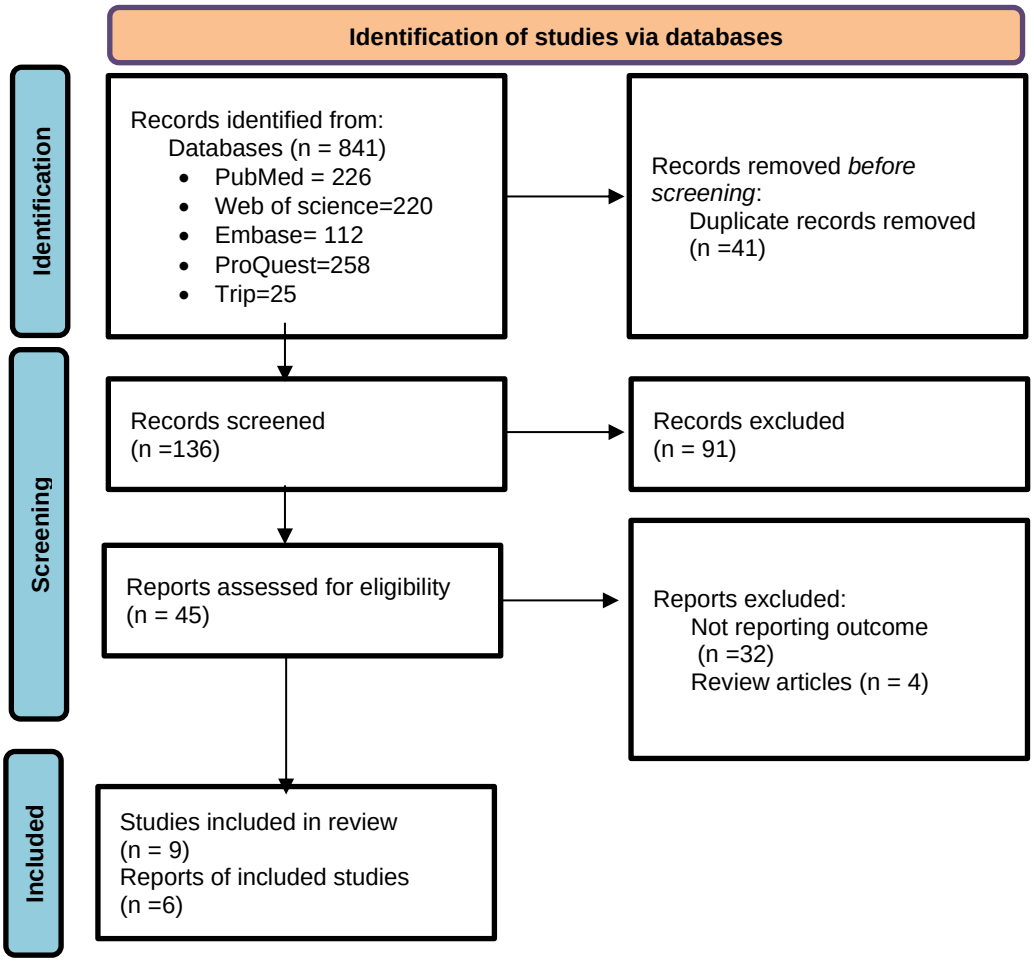

Figure 1: Flowchart for systematic review

Risk of bias

The Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) tool was used to determine the risk of bias in selected articles (21). Two authors independently evaluated the studies for bias, following the guidelines of the 2014 SYRCLE risk of bias tool. The senior authors were responsible for resolving disputes of opinions. The SYRCLE tool includes ten domains to check biases of selection, performance, detection, attrition, and reporting. Each domain was scored as low, high, or unclear. The response of "low bias" indicates that the risk of bias is low, and "high bias" defines a high risk of bias. Items that are not stated clearly and directly in the method of the articles are considered "unclear."

Data analysis

The included studies were first grouped based on outcomes measures (ABR threshold) and cochlear regeneration. Meta-analysis was conducted using Stata software (17, Stata Corp LLC, College Station, USA). It was based on fixed effect models and reported in a forest plot. The mean difference between intervention and control groups was considered as the estimated effect size for hearing function following stem cell injection. Subgroup analysis was done with fixed effect

models for extracted data as follows: Animal species (mice, rat, guinea pig, & pig), noise exposure (pure tone, wideband noise, & impulse), type of ABR (click & frequency), type of graft (allograft & xenograft), time of cell injection after auditory injury (equal and less than 2 days, more than 2 days), route of injection (vein, cochleostomy, round window, posterior semicircular canal, & subarachnoid) and type of injection (systemic & local), type of cell (mesenchymal stem cell, neural stem cell, & epithelial stem cell), and total dose of stem cell in three groups (less than 1000, more than one million and between those). Furthermore, the frequency-ABR was divided to two groups for analyzing: Low frequencies included tone bursts of 1, 2, 4, 6, 8 KHz and click and high frequencies consisted of tone bursts of 12, 16, 20, 24 and 32 KHz).

The heterogeneity index, I^2, the variability between studies was evaluated. It was 50% or more, meaning that variability of effect size is due to significant heterogeneity between studies. Therefore, subgroup analysis was applied based on some variables. The funnel plot and statistical Egger's test were used to determine publication bias. In order to sensitivity analysis, different meta-analysis models, including the fixed-effect model and the random -effect model were used for estimating the mean difference between two groups. All of the analyses were considered statistically significant based on a p-value less than 0.05.

Results

Study selection

In this review, 841 studies have identified with the database searching. Following the title and abstract screening, 45 studies were completely evaluated for eligibility, and 36 studies were excluded for not meeting inclusion criteria or to be review articles. Finally, nine studies remained for data extraction and six of them were analyzed (Figure 1).

Study characteristics

Noise exposure characteristics

The wideband noise and pure tone were the most common noises to create a NIHL model (Table 1). Only a study used impulse noise (19). The noise intensity in the studies was 110 to 120 dB SPL for different species. The average time exposure was 14.12 ± 23.72 hours and the most time was 72 hours continuously with the intensity of 112 dB SPL in the study's Parker (22). A study used impulse noise for eight times continuously in minipigs (19). Table 2 shows information of included studies.

Table 1. Characteristic of some variables among included studies (n = 9)

Variables	Number (%)
Type of noise	
Wideband noise	4 (44.4)
Pure tone	4 (44.4)
Impulse noise	1 (11.2)
Source of stem cell	
Human	4 (44.4)
same species	4 (44.4)
Other	1 (11.2)
Type of stem cell	
Mesenchymal stem cell	5 (55.6)
Neural stem cell	2 (22.2)
Epithelial stem cell	1 (11.1)
Hematopoietic Stem Cell	1 (11.1)
Route of cell injection	
Systemic injection	2 (22.2)
Local injection	7 (77.8)
Cochleostomy	3 (42.8)
Round window	2 (28.6)
Posterior semicircular canal	1 (14.3)
Subarachnoid cavities	1 (14.3)
Hearing assessment	
Click-ABR	3 (33.3)
TB-ABR	5(55.6)
DPOAE	1(11.1)
Total dose of stem cell	
Less than 10^3	1(11.2)
$10^3 - 10^6$	4 (44.4)
More than 10^6	4 (44.4)
Time of cell injection	
≤ 2 days	7 (87.5)
>2 days	1(12.5)
Time assessment after cell injection	
≤ 14 days	3 (37.5)
>14 days	5 (62.5)
Histological evaluation	
Yes	8 (88.9)
No	1(11.1)

Confirmation of NIHL

Some studies reported persistent hearing loss using histological assessment and auditory threshold (9, 18, 23, 24). Several studies only reported cochlear histopathological findings (8, 22). Auditory function was measured by click-ABR (9, 19, 23), frequency-specific ABR (17-19, 24), and DPOAE (12). The frequency-specific ABR included 1, 2, 4, 8, 16, 20, 24, 32 KHz. The follow-up time of permanent threshold shift (PTS) or tissue damage after noise exposure was not similar between the studies and its mean was 12.6 ±9.6 days and some studies had not mentioned proof time of the PTS (9, 12, 17).

Stem cell characteristics

The most common of the injected stem cells was the mesenchymal stem cell (Table 1). Other used adult stem cells included neural stem cell (18, 22), epithelial stem cell (23) and mononuclear cell as hematopoietic stem cell (8) (Table 2). The source of stem cells was various; three studies were prepared from human

pregnancy products, such as umbilical cord blood (8) and Wharton's jelly (17, 19). Other cases were from bone marrow (9, 12), adipose tissue (24), epithelium of the nose (18), tongue (23), and cerebellar cells of animals (22). All of them had one-time injection and the dose of stem cell was different based on animal species and route of injection. The mean dose of cell was $2.8 \times 10^8 \pm 4.6 \times 10^8$ that minimum dose was 10^2 cells local injection in rat (18), and the maximum dose was 10^9 subarachnoid injection in minipigs (19). The volume of injection was different, so that intravenous injection was 300 microliter (9). The minimum and maximum volumes were 0.1 microliter (18) and 10 milliliters (19).

Several studies were related to xenografts (8, 9, 17, 19, 22). MSCs isolated from human umbilical cord were injected into deafened mice (17), rat (9) and minipigs (19) without using immunosuppressive drugs, and no graft rejection reported during 3-4 weeks. Parker et al. injected neural stem cell from murine to guinea pig and used cyclosporine 16 mg/kg intraperitoneal for two days before and 6mg/kg oral for six weeks after cell injection (22). Another study used hematopoietic stem cells in NOD-SCID mice, eliminating the need for immunosuppressive drug administration (8).

The average time for stem cell injection was 2.3 ± 1.9 days (range: 1 to 7 days). The most common time was two days after noise exposure (8, 9, 17, 19, 23). Other studies were injected during 24 hours (18, 22, 24) and seven days after noise damage (19). The most abundant route of cell injection was direct injection to scala tympani through round window (12, 24) or lateral wall of the cochlea using a cochleostomy (18, 22, 23). None of these studies reported further damage to the auditory system after surgery and this injection method was safe.

Two studies had intravenous injection and migration into cochlea (8, 9). Some studies had cell injection through the posterior semicircular duct (17) and subarachnoid (19) without additional damage and reported migration of stem cells into the cochlea.

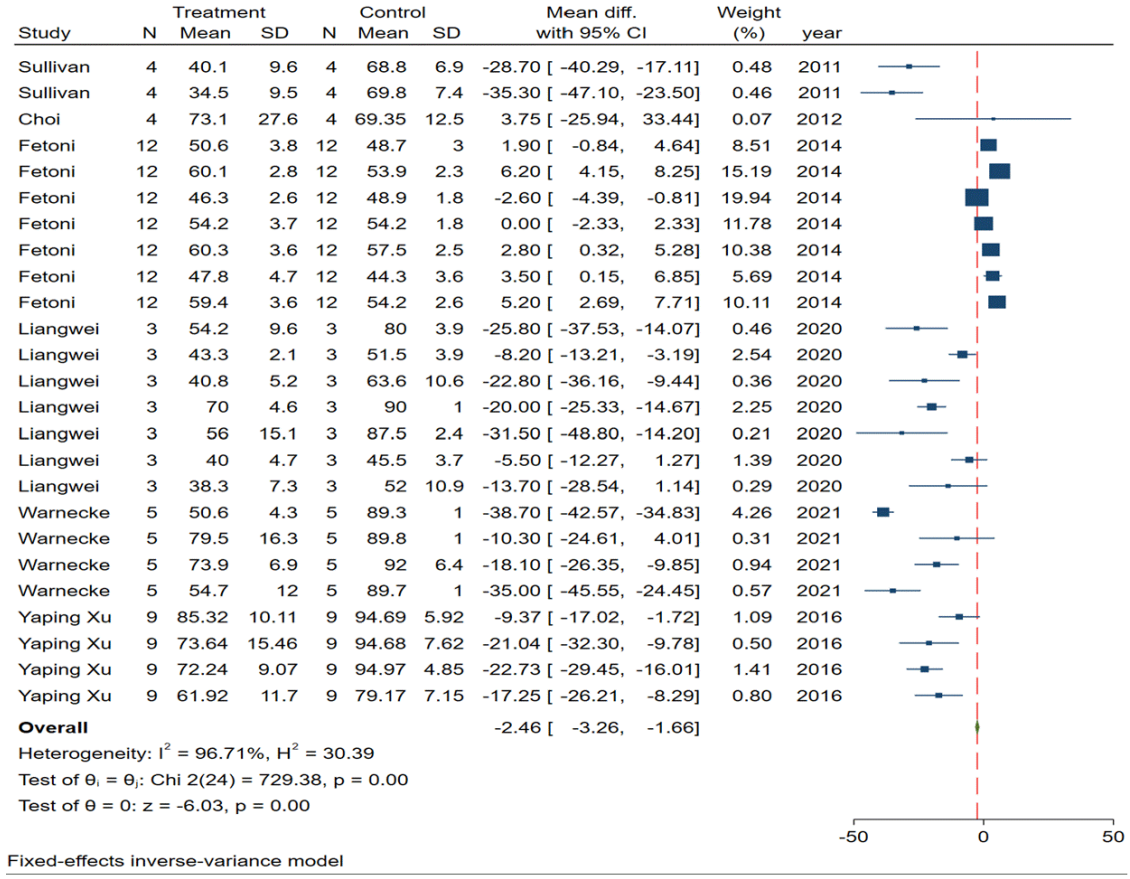

Figure 2: Effect size of stem cells injection on hearing threshold in Auditory Brainstem Response

Outcome measurement

The average time for evaluating the results was about 26.6 ± 17.3 days after transplantation; the maximum and minimum were about 60 days (8) and 7 days (24). Only one study has not mentioned this time (12). In most studies, ABR and histology were used for

auditory system evaluation (9, 18, 19, 23, 24). One study used distortion product otoacoustic emission to assess outer hair cell function and did not report histological findings (12). Two studies had only histological findings without reporting of auditory threshold (8, 22).

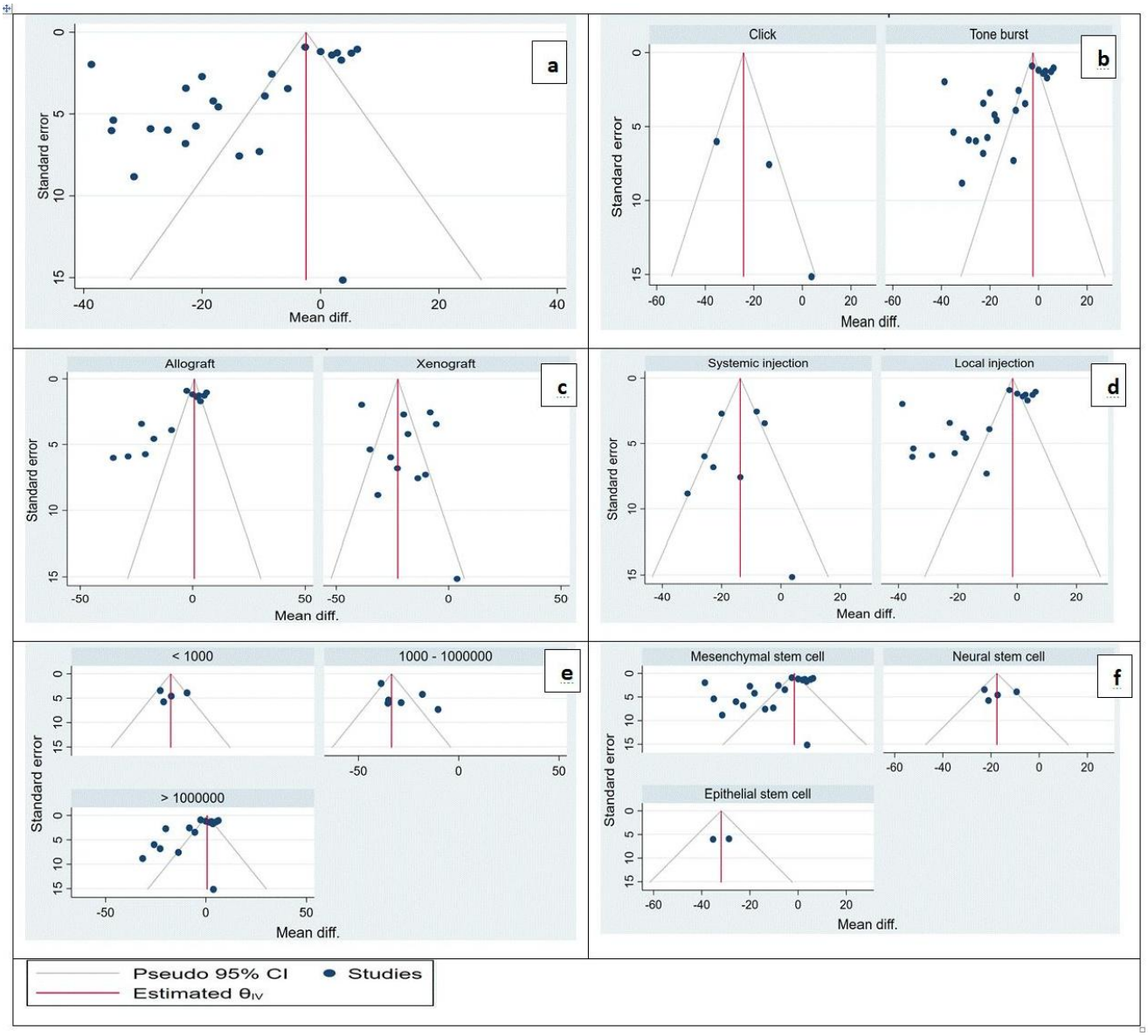

Figure 3: Funnel lot for publication bias of six studies based in a: threshold of ABR, b: type of ABR, c: type of transplantation, d: site of injection, e: cell density, f: type of cell injection.

Effect of stem cell injection on auditory system

No histological integration was found in the sensory epithelium of the organ of Corti in the studies. Some studies reported improvement of the ABR threshold after stem cell injection (17-19, 23). A study reported a slight improvement of the ABR threshold (24), but was statistically insignificant. The findings of the meta-analysis show a significant mean difference between intervention and control groups, Figure 2 (effect size: -2.46, 95% CI: -3.26, -1.66). The heterogeneity of the six studies was large (I^2: 96.7), so subgroup analysis was done. This study used different stratifications based on animal species, noise exposure, type of ABR, frequency-ABR, type of graft, time, site and type of injection, type of cell, and total dose that were summarized in Table 3.

A significant mean difference was observed in the click-ABR threshold that the heterogeneity of the studies was less than high frequency tones (I^2: 77.08, vs 96.99). The present study found a significant difference in xenograft group (effect size: -22.63, $p< 0.05$) compared to allograft group (effect size: 0.74, $p > 0.05$). Additionally, this research found significant mean differences with narrow confidence intervals for local injection (n = 148) and MSC injection (n = 129), but a high heterogeneity in those studies (Table 3).

Table 2. Summary of included studies for review

Study	NIHL induction (Noise exposure)				Animal characteristics			Intervention characteristics						Primary outcome	Secondary outcome
	Type of noise	Noise duration	Noise level(dB SPL)	Side	Species	Age (week)	Sex	Cell type	Source	Total dose (n)	Route of delivery	Delivery time after noise (days)	Assessment time after injection (days)	Remission of Hearing function	Integration in OC
Warnecke, 2021. (17)	16 KHz	4 h	118	Left	Mice (C57BL/6)	4	F	MSC [1]	Human	$1 * 10^3$	Posterior semicircular canal	2	14	Yes	NA
Revoltella, 2008. (8)	8 KHz	5 h	120	Both	Mice (Nod-scid)	8	NA	HSC[2]	Human	$2.5 * 10^5$	Vein	2	60	NA	Yes
Sullivan, 2011. (23)	1-80 KHz	2 h	120	Both	Mice (CBA/caH)	6	F, M	ESC[3]	Mice	$2 * 10^3$ $4 * 10^3$	Cochleostomy	2	28	Yes	No
Ya-ping Xu, 2016. (18)	10 KHz	2 h for 7d	110	Both	Rat (Sprague-Dawley)	4	M	NSC[4]	Rat	$1 * 10^2$	Cochleostomy	1	20	Yes	NA
Fetoni, 2014. (24)	6 KHz	1 h for 5 d	120	Both	Guinea pig	12	NA	MSC (ASC [5])	Guinea pig	$1.5 * 10^6$	Round window	1	7	Yes	No
Choi, 2012. (9)	0.5- 20 KHz	9 h	120	Both	Rat (Sprague-Dawley)	8	M	MSC	Human	$4 * 10^6$	Vein	2	28	No	No
Liangwei, 2020. (19)	Impulse noise	80 times	NA	Both	Minipig	4	NA	MSC	Human	$1 * 10^9$	Subarachnoid	7	14	Yes	No
Parker, 2007. (22)	1-4 KHz	72 h	112	Both	Guinea pig	4	M	NSC	Murine	$1.5 * 10^6$	Cochleostomy	1	42	NA	No
Ajalloueyan, 2013. (12)	8-16 KHz	2 h	120	Both	Rat (Sprague-Dawley)	NA	M	MSC	Rat	$4 * 10^5$	Round window	NA	NA	NA	NA

1: Mesenchymal stem cell, 2: Hematopoietic stem cell, 3: Epithelial stem cell 4: Neural stem cell 5: Adipose-derived stem cell, NA: Not available, F: female, M: male, h: hour, d:day, OC: organ of Corti

Several factors are implicated in all the heterogeneity, including animal species, type of stem cell, route of delivery, assessment of auditory function, and ABR frequency. In the sensitivity analysis, the analysis models yielded results in the same direction, although the estimated effect sizes differed, as expected given the heterogeneity among the studies. A few studies have reported cell migration into the cochlea after stem cell injection. The stem cells have been traced in lateral wall and spiral ligament (23), spiral ganglion neurons (SGN) and vessels (18, 22). Two studies demonstrated cell migration to the organ of Corti and SGNs, but did not assess auditory function with ABR (8, 22). Fetoni et al. observed an accumulation of injected cells as a mass in the tympanic scala and found few cells in the vestibule scala (24). Three studies reported cell migration into SGNs (9, 18, 22). Ya-ping et al. observed the recovery of the ABR threshold (18), whereas choi et al. did not find a threshold improvement (9). Another study did not use ABR (22).

Liangwei et al. used subarachnoid injection of stem cell in minipigs and found cells in cochlea vessels, bony wall of tympanic and scala vestibuli, not the organ of the Corti (19). Two studies did not have an immunohistochemistry assessment (12, 17).

Risk of bias

Table 4 shows the results of bias assessment based on the SYRCLE tool. From nine studies, only two studies (18, 24) reported random group allocation. Almost 77.7% (n = 7) of the studies had similar animals at baseline. The outcomes of 66.6% studies (n = 6) were not evaluated randomly and blinded, and the rest were unclear. Only two studies (22, 23) had the reporting of the mortality rate and one study had the reporting bias (23).

Table 3. The estimated effect size of hearing function based on subgroup analysis

Variable	Number of studies	Number of animals	Mean difference 95% CI	Weight (%)	I^2 (%)	p-value
Species						
Mice	2	28	-33.49 (-36.50, -30.47)	7.02	84	0.001
Rat	2	40	-17.13 (-21.19, -13.07)	3.87	55.46	<0.05
Guinea pig	1	84	1.96 (1.07, 2.84)	81.60	88.41	<0.05
Pig	1	21	-13.89 (-16.88, -10.97)	7.51	76.52	<0.05
Noise exposure						
Pure tone	3	130	-1.22 (-2.06, -0.39)	91.48	97.61	<0.05
Wide band noise	2	12	-29.37 (-37.34, -21.41)	1.01	65.29	0.06
Impulse	1	21	-13.89 (-16.81, -10.98)	7.51	76.52	<0.05
Type of ABR						
Click-ABR	3	9	-24.23 (-33.05, -15.41)	0.82	77.08	<0.05
Frequency- ABR	5	162	-2.28 (-3.08, -1.47)	99.18	96.99	<0.05
Frequency_ABR						
Low frequency	6	90	-1.36 (-2.43, -0.29)	55.39	97.73	<0.05
High frequency	5	83	-3.82 (-5.02, -2.62)	44.61	93.21	<0.05
Type of graft						
Allograft	3	128	0.74 (-0.12, 1.60)	86.35	94.09	0.09
Xenograft	3	45	-22.63 (-24.79, -20.47)	13.65	92.07	<0.05
Type of injection						
Systemic	2	25	-13.72 (-16.63, -10.12)	7.57	73.98	<0.05
Local	4	148	-1.53 (-2.36, -0.70)	92.43	97.50	<0.05
Type of cell						
MSC	4	129	-1.57 (-2.39, -0.75)	95.26	97.08	<0.05
NSC	1	36	-17.53 (-21.63, -13.43)	3.80	57.41	<0.05
ESC	1	8	-31.94 (-40.21, -23.67)	0.94	0.0	<0.05
Route of injection						
Vein	1	4	3.75 (-25.94, 33.44)	0.07	0.0	0.80
Cochleostomy	2	42	-20.37 (-24.04, -16.70)	4.74	70.63	<0.05
Round window	1	84	1.96 (1.08, 2.85)	81.6	88.41	<0.05
Posterior semicircular canal	1	20	-33.72 (-36.96, -30.48)	6.08	90.16	<0.05
Subarachnoid	1	21	-13.89 (-16.81, -10.98)	7.51	76.52	<0.05
Time of cell injection						
≤ 2 days	5	152	-1.53 (-2.36, -0.70)	92.49	97.34	<0.05
>2 days	1	21	-13.89 (-16.81, -10.98)	7.51	76.52	<0.05
Total dose of stem cell						
Less than 10^3	1	36	-17.53 (-21.63, -13.43)	3.80	57.41	<0.05
$10^3 - 10^6$	2	28	-33.49 (-36.50, -30.47)	7.02	84.00	<0.05
More than 10^6	3	109	0.63 (-0.22, 1.47)	89.18	92.28	0.146

MSC: Mesenchymal stem cell, NSC: Neural stem cell, ESC: Epithelial stem cell, Low frequency: Click, 1, 2,4,6,8 KHz, High frequency: 12, 16, 20, 24, 32 KHz

Publication bias

The six studies for meta-analysis were investigated regarding publication bias using funnel plot and Egger test. Figure 3 shows asymmetry among studies based on ABR threshold and some subgroups. The findings of the Egger test were less than 0.05 and confirmed the publication bias. The reasons of this bias can be related to 1) Some animal studies have not reported statistical findings and have focused to histological findings (8, 22), 2) Studies with negative findings have little chance of being published and are usually rejected by journals, 3) This study had a literature review based on keywords and English language, and 4) The variability existed between studies overall, as well as subgroups.

Discussion

Hearing loss of noise exposure is PTS when a severe and irreversible cochlear damage exists (2). The proving of PTS is done by histological and functional assessment in animal studies (25). The proof time of it can be between two to six weeks, and some studies have demonstrated irreversible damage or hair cell loss during three weeks after noise exposure (26-28). In this review, the lowest time was seven days (18, 24) and the most time was 12 to 62 days in Revoltella's study (8) and 30 days in Sullivan's study (23).

ABR is the best tool for achieving information of auditory function, reflecting the integrity of the auditory pathway from the sensory epithelium of the

cochlea to the brain stem in response to sound stimulation (29). All of the investigated studies except three studies reported auditory threshold using ABR in different time frames (8, 12, 22).

Table 4. Risk of Bias for included studies

Author	Year	Random group allocation	Group similar at baseline	Blinded group allocation	Random housing	Blinded interventions	Random outcome assessment	Blinded outcome assessment	Reporting of drop-outs	Selective outcome reporting	Other biases
Revoltella	2008	High	Low	High	Unclear	Unclear	Unclear	Unclear	High	Low	Low
Sullivan	2011	High	Low	Unclear	Unclear	High	Unclear	Unclear	Low	High	Low
Warnecke	2021	High	Low	Unclear	Unclear	High	Unclear	Unclear	Unclear	Low	Low
Ya-ping Xu	2016	Low	Low	High	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Low
Fetoni	2014	Low	Low	High	Unclear	High	Unclear	Unclear	Unclear	Unclear	Unclear
Choi	2012	Unclear	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Low
Liangwei	2020	Unclear	Unclea	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Low
Parker	2007	Unclear	High	High	Unclear	High	Unclear	Unclear	Low	Low	Unclear
Ajalloueyan	2013	Unclear	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Low	Unclear

Cell homing is crucial in stem cell transplantation, depending on the number of injected cells, site of injection, secretion of inflammatory mediators, and cytokines for cell calling in target tissue (11). The different signaling pathways exist for mobilization and replacement of cells in target tissue. One of those is the SDF1-CXCR4 pathway, which has been proven in neovascularization and neurogenesis (30, 31). Stromal cell-derived factor-1(SDF1) is a chemokine expressed in damaged tissue such as liver, lung, skin, bone marrow, and cochlea (30, 31). The main receptor of SDF1 is a member of G-protein receptor family as CXCR4. This protein ligand is expressed on the surface of most cells and types of stem cells (32). This signaling pathway has a critical role in promoting cell migration to damaged cochlear tissue (33).

The number of cells injected has varied between studies due to the injection site and size of the animal model. The minimum dose was 100 cells in 0.1 microliter via cochleostomy (18), which was little compared to similar studies (22, 23). Those cells were neural stem cells that secrete neurotrophic factors and were probably effective in remission of hearing function (18).

A large volume of cells is required for the systemic injection due to the trapping of cells in the lung tissue. A study has used a 300-micro-litre suspension of MSC with total cell number 4×10^6 via intravenous injection (9).

The present study found no reporting of graft rejection in the xenograft studies. Most transplanted cells were MSCs, having a low risk of rejection compared to other stem cells due to the immune system regulatory feature (3). In the rest of some studies, the immune system of animals had been suppressed genetically (8) or by administrating immunosuppressive drugs like cyclosporine (22).

The findings of the meta-analysis show a decrease in the mean of the ABR threshold after cell injection, but should be interpreted with caution due to the high heterogeneity of studies. In subgroup analysis, a significant mean difference was found in xenograft studies. Human cells appear to possess regenerative capacity and may thus represent a promising avenue for future clinical investigations (34). Although allograft studies carried greater statistical weight, they did not demonstrate significant findings. This may be attributed to the results of Fetoni's study, which failed to show improvement in hearing thresholds, potentially due to cell accumulation within the labyrinthine fluid and the absence of effective cell migration into cochlear tissue (24).

This study did not find hearing improvement along with hair cell regeneration or integration in the studies (18, 23). The injected cells have been detected in other parts of the cochlea, such as stria vascularis, lateral wall of cochlea, and spiral ganglion. The tracing time varied across the studies. Most of it related to the Revoltella's and Parker's studies almost 50 days (8, 22), which had no auditory evaluation. Two studies had no similar ABR threshold with cell migration to the spiral ganglion area (9, 18). One study had no evidence of hearing threshold improvement (9), while another study showed improvement in middle and high frequencies (18) due to secreted neurotrophic factors from stem cells. The positive effect on high frequency area is explainable by increased blood supply and high concentration of factors at the base of the cochlea.

Two studies reported auditory threshold improvement without cell migration into cochlear tissue. It could be due to increased expression of anti-apoptotic genes after cell injection in this environment, which may promote survival and protection of remaining epithelial cells in the organ of Corti (17, 23).

The current analysis shows a pooled effect size of -2.46 dB for hearing threshold improvement in ABR testing. The systemic delivery of stem cell (subarachnoid or vein) was superior to local injection

(-13.72 dB and -1.53 dB). This finding is consistent with Chorath's study (14), but given the two studies analyzed, no definitive conclusion can be drawn about it. Intravenous injection appears to be an effective and safe route of cell delivery. A clinical study reported the safety of intravenous injection of stem cells in children with SNHL, showing ABR threshold reduction of approximately 5 to 30 dB at frequencies of 0.5, 1, and 2 kHz (34).

The heterogeneity observed across the included studies and their subgroups necessitates a cautious interpretation of these findings. Evidence of publication bias was identified through funnel plot asymmetry and Egger's test (p-value < 0.05). While a low risk of bias was noted for baseline group similarity and outcome reporting, the majority of included studies presented an unclear risk of bias across other domains. Limitations of this review included the small number of experimental studies with NIHL models, dissimilarity in methodological protocols for cell injection and outcome assessment and the use of articles in the English language.

In Conclusion

Improving the ABR threshold after cell injection indicates that mechanoelectrical transduction has occurred in cochlea and the neural signal has been transmitted by auditory nerve fibers to higher levels of the auditory pathway in the brainstem. Despite the limitations and high heterogeneity among the studies in this review, MSCs, even of human origin and systemic injection, can be effective in functional integrity in the organ of Corti and repairing of the auditory system without serious complications. Therefore, more studies are recommended with the above-mentioned findings to achieve strong evidence for clinical studies.

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Authors' Contributions

All authors contributed to the design of the study, data acquisition, data interpretation, drafting manuscript, critical revision of manuscript, and final approval and accountability. Data analysis was conducted by Mahbobeh Oroei.

Conflict of Interest

None.

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