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Stem cell transplantation in noise induced hearing loss☆

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ABSTRACT

Objective: To investigate efficacy of bone marrow stem cell implantation in rehabilitation of noise induced hearing loss in rats.
Materials and methods: Hearing loss was induced in male rats by a continuous wide-band noise (8–16 kHz/120 dB/120 min). Ten microliter of stem cell containing solution was injected by a Hamilton syringe with 30G needle through the round window membrane. Hearing status was examined by, distortion product otoacoustic emissions using DP-OAE. Animals were studied in 4 different groups: (1) Normal hearing animals, undergoing sham surgery (no injection done, only round window membrane ruptured and sealed). (2) Deaf animals, undergoing sham surgery. (3) Deaf animals undergoing surgery and injection of solvent (artificial perilymph). (4) Deaf animals undergoing surgery and injection of artificial perilymph containing BMSCs.
Results: DP-Gram in rat with normal hearing undergoing sham surgery show that procedure has neither negative impact on normal cochlear nor on deaf cochleas. No significant difference ($p = 0.25$) between ears excludes artificial perilymph as a confounding factor. There is no significant difference between ears in animals receiving BMSCs.
Conclusions: Implanted cells with normal histologic structures have no physiologic function and hearing rehabilitation. Further studies by monitoring the survival of these cells with histologic and appropriate biomarkers will help to investigate differentiation process of these cells.

1. Introduction

Hearing loss due to noise exposure was described in the 18th century for the first time [1]. Noise induced hearing loss (NIHL) is one of the most common work related morbidities. Acoustic trauma has a great impact on health systems. Iranian government pays unlimited amount of salaries and social preferences for victims of acoustic war injury. US navy pays 300 million$ each year on rehabilitation and treatment of NIHL [2]. The important point is that most of the victims of NIHL are young people, and on the other hand our ability to treat this morbidity is very limited.

Stem cell implantation opened a new horizon in treating age related or disease induced cell loss [3]. There are evidences showing that otic sac stem cell can produce different kinds of cells in the cochlea [4]. Also, after hair cell injuries, it seems that supporting cells can differentiate to hair cells [5].

There are studies regarding stem cell transplantation in hearing rehabilitation [6,7], but their efficacy in treatment of acoustic trauma is unknown and controversial.

In order to investigate efficacy of bone marrow stem cell in rehabilitation of NIHL in rats this study was performed.

2. Materials and methods

Male rats (Sprague-Dawley species) weighting 200–250 g were used. For anesthesia, intra-peritoneal injection of 0.1 cm^3/100 g ketamine 15% and 0.5 cm^3/100 g diazepam 10 mg/2 cm^3 was used. In order to have access to round window, a posterior tympanotomy was used preserving the facial nerve (Fig. 1). For stem cell injection a Hamilton syringe with 30G needle was used. Before injection 5 μl of perilymph was aspirated and 10 μl of stem cell containing solution was injected. In order to seal the round window membrane a free muscle graft was used. At the end of the procedure, trauma to the tympanic membrane and ossicular chain or middle ear blood accumulation was ruled out by trans-canal examination, and in all of the cases middle ear was aerated at the end of the procedure.

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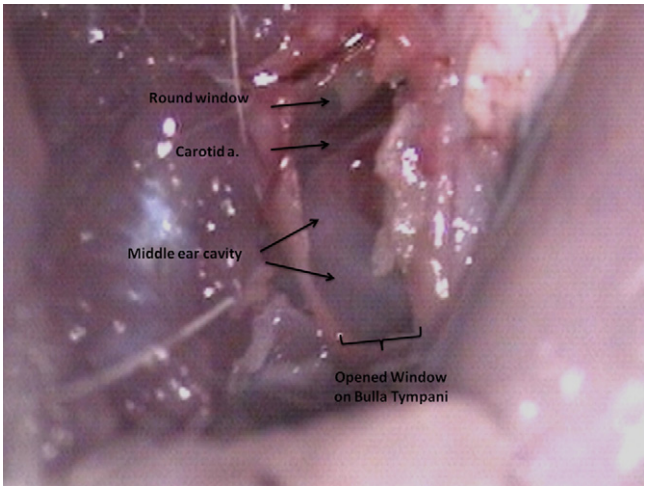

Fig. 1. Middle ear cavity at the end of dissection. Carotid artery and its relation to round window is shown.

Table 1
OAE results in rats with normal hearing.

DP-OAE	Mean	SE	SD	Min	Max
DP-Gram					
2 kHz	12.761	.7380	7.9489	1.9	37.6
3 kHz	22.754	.8889	9.5736	6.6	42.3
4 kHz	27.250	.7680	8.2720	6.1	42.2
6 kHz	31.528	.7684	8.2760	11.4	49.3
8 kHz	32.378	.7258	7.8173	9.0	48.1
DP-IO 6 kHz					
50 dB	27.449	.6280	6.7632	7.3	41.3
55 dB	30.854	.6739	7.2579	2.1	42.5
60 dB	34.993	.7002	7.5415	6.2	47.7
65 dB	36.527	.6778	7.3003	6.1	50.1
70 dB	33.237	.5878	6.3310	9.0	47.6
DP-IO 8 kHz					
50 dB	36.547	.7632	8.2200	.7	46.9
55 dB	38.388	.6634	7.1447	6.2	49.3
60 dB	37.774	.6155	6.6289	6.4	54.1
65 dB	40.159	.8933	9.6211	7.1	56.2
70 dB	42.490	1.0286	11.0783	−.4	56.0

SE: standard error; SD: standard deviation.

In order to confirm intra-cochlear injection, a fluorescent dye was injected trough round window in the cochlea (Fig. 2).

It was shown that a wide band noise with 116 dB sound pressure level for 120 min can induce permanent threshold shift in rats [8]. In order to induce NIHL a continuous wide band noise frequency range 8–16 kHz was used with the intensity of 120 dB for 120 min. Each animal was exposed to noise in a separate cage. The noise level was check trough the exposure with sound level meter to prevent under-threshold exposure.

Hearing status was examined by, distortion product otoacoustic emissions using DP-OAE; Capella, Madsen; Denmark (Table 1).

2.1. Bone marrow stem cell preparation

We harvested BMSCs from femur bone marrow. Cells were cultured in α-MEM medium. Penicillin/streptomycin and amphotericin was added to prevent microbial overgrowth. After 3–4 passages cells were checked to be alive and anti-CD44, anti-CD45, and anti-fibronectin antibodies were used to approve these cells to be stromal. For injection 4×10^5 cells were prepared in 10 μl.

2.2. Study groups

Animals were studied in 4 different arms:

(1) Normal hearing animals, undergoing sham surgery (no injection done, only round window membrane ruptured and sealed).
(2) Deaf animals, undergoing sham surgery (no injection, only round window membrane ruptured and sealed).
(3) Deaf animals undergoing surgery and injection of solvent (artificial perilymph).
(4) Deaf animals undergoing surgery and injection of artificial perilymph containing BMSCs.

Interventions were done on the left side, and right side of all cases was studied as the control.

Statistical Package for Social Sciences (SPSS; version 16.0) was used for data analysis.

3. Results

Eight animals studied in each group. OAE threshold change in different groups are shown in Table 2. DP-Gram in rat with normal hearing undergoing sham surgery show that procedure has no negative impact on cochlear function. Difference of OAE threshold are not statistically different between two ears, with and without intervention (8 Hz, 70 dB: $p = 0.6$). Sham surgery has neither any impact on deaf animals (Fig. 3). DP-Gram in 8 kHz show no statistically significant difference ($p = 0.46$).

Regarding threshold shift in control and intervention ears of animals undergoing artificial perilymph injection, no significant difference ($p = 0.25$) between ears excludes artificial perilymph as a confounding factor.

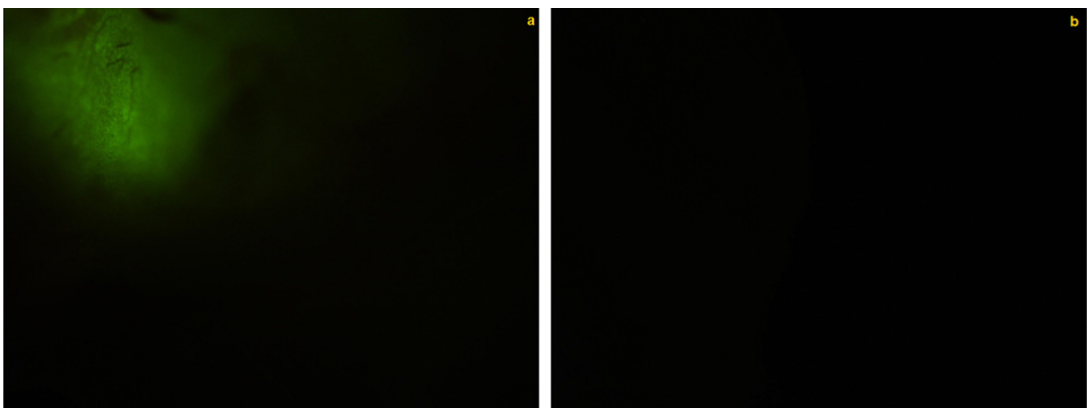

Fig. 2. (a) Positive fluorescent in the ear with injection of dye. (b) Control ear with no injection.

Table 2
OAE results in different groups of animals. For each group intervention and control ear is shown.

DP-OAE	Normal[a]		Sham[b]		Perilymph[c]		BMSC[d]	
	Case	Control[e]	Case	Control	Case	Control	Case	Control
DP-Gram								
2 kHz	−0.2	1.4	3	−0.3	0.55	−0.8	−3.89	0.99
3 kHz	0.6	−5.4	−6.7	−2	−0.1	0	−5.38	0.28
4 kHz	−4.4	−0.7	−8.5	−2.4	−0.25	0.15	−0.51	2.49
6 kHz	−2.45	7	−5.2	3	0.25	−0.3	−3.69	−4.13
8 kHz	−2.95	−1.65	7.9	8.8	−0.2	−0.75	−0.35	−1.1
DP-IO 6 kHz								
50 dB	−2.55	3.35	−1.2	1.3	1.05	−0.7	1.63	−0.4
55 dB	−1.75	2.3	−1.1	1.4	0.2	−0.05	−0.94	−1.09
60 dB	−4.3	2.7	1.2	3.7	0.25	0.05	−1.89	−1.91
65 dB	−4.35	−1.85	−6	4.9	−0.55	−0.35	−1.49	−1.74
70 dB	0.35	−3.55	−5.2	5.7	−0.4	−0.35	−1.3	−0.93
DP-IO 8 kHz								
50 dB	0.35	2.7	0.1	1.3	0.1	−0.6	3.6	1.63
55 dB	−14.3	0.45	−0.2	5.6	−0.45	−0.3	−0.34	3.34
60 dB	−0.5	−2.4	−2.1	6.2	0.45	−0.55	−2.73	−0.76
65 dB	0.1	−9.88	−1.9	7.1	0.25	−0.6	−2.56	2.68
70 dB	−4.2	0.92	0.4	4.3	−0.9	−0.95	−6.11	0.79

[a] Normal hearing rats undergoing surgery with no injection.
[b] Deaf rats undergoing surgery with no injection.
[c] Deaf rats undergoing surgery with artificial perilymph injection.
[d] Deaf rats undergoing surgery with bone marrow stem cell.
[e] Intervention is done in one ear in each rat and the other ear is control.

As is shown in Fig. 4 there is no significant difference between ears in animals receiving BMSCs. Threshold shift values and *p* value for different frequencies is provided in Table 3. Although DP-IO is significant in 8 kHz–70 dB, but this difference does not seem to be significant in audiologic point of view.

4. Discussion

In order to achieve the best possible result from stem cell transplantation the cells used should be: easily harvested; easily proliferated in the culture media; neutral immunologically; able to survive long-term in the host tissue. Therefore, BMSCs seem to be an appropriate option in this regard [9]. On the other hand, their easy harvest makes autologous transplantation possible and eliminates ethical issues concerning neural or fetal stem cells [10]. The exact mechanism these BMSCs help injured tissues is controversial; some hypothesize that these cells produce cytokines and necessary factors for tissue repair and assume them as a small molecular factory for trophic agents [11], and some other believe these cells differentiate to the target cells and replace the original cells [9].

However, as our results show, BMSCs injected in the cochlea was not able to produce any physiologic function. There may be some explanation for this. First, they may have not differentiated to the hair cells to be able to make contact with the cochlear nerve and produce auditory signals. These BMSCs are shown to have the potential of differentiation to various kinds of cells, depending on the context tissue. It is shown that pluripotent stem cells can differentiate to spiral ganglion and can make contact with the cochlear hair cell [12]. However, Ito et al. did not

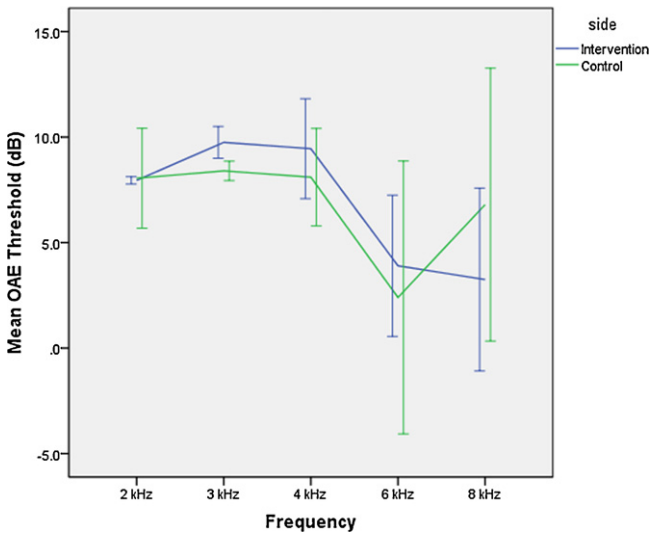

Fig. 3. OAE results in noised induced deaf animals undergoing perilymph injection.

Table 3
Threshold shift for different frequencies in deaf animals after BMSC injection in intervention and control side.

DP-OAE	Case	Control	*p* value
DP-Gram			
2 kHz	−3.89	0.99	0.06
3 kHz	−5.38	0.28	0.13
4 kHz	−0.51	2.49	0.27
6 kHz	−3.69	−4.13	0.91
8 kHz	−0.35	−1.1	0.71
DP-IO 6 kHz			
50 dB	1.63	−0.4	0.3
55 dB	−0.94	−1.09	0.93
60 dB	−1.89	−1.91	0.99
65 dB	−1.49	−1.74	0.89
70 dB	−1.3	−0.93	0.86
DP-IO 8 kHz			
50 dB	3.6	1.63	0.42
55 dB	−0.34	3.34	0.3
60 dB	−2.73	−0.76	0.65
65 dB	−2.56	2.68	0.25
70 dB	−6.11	0.79	0.04

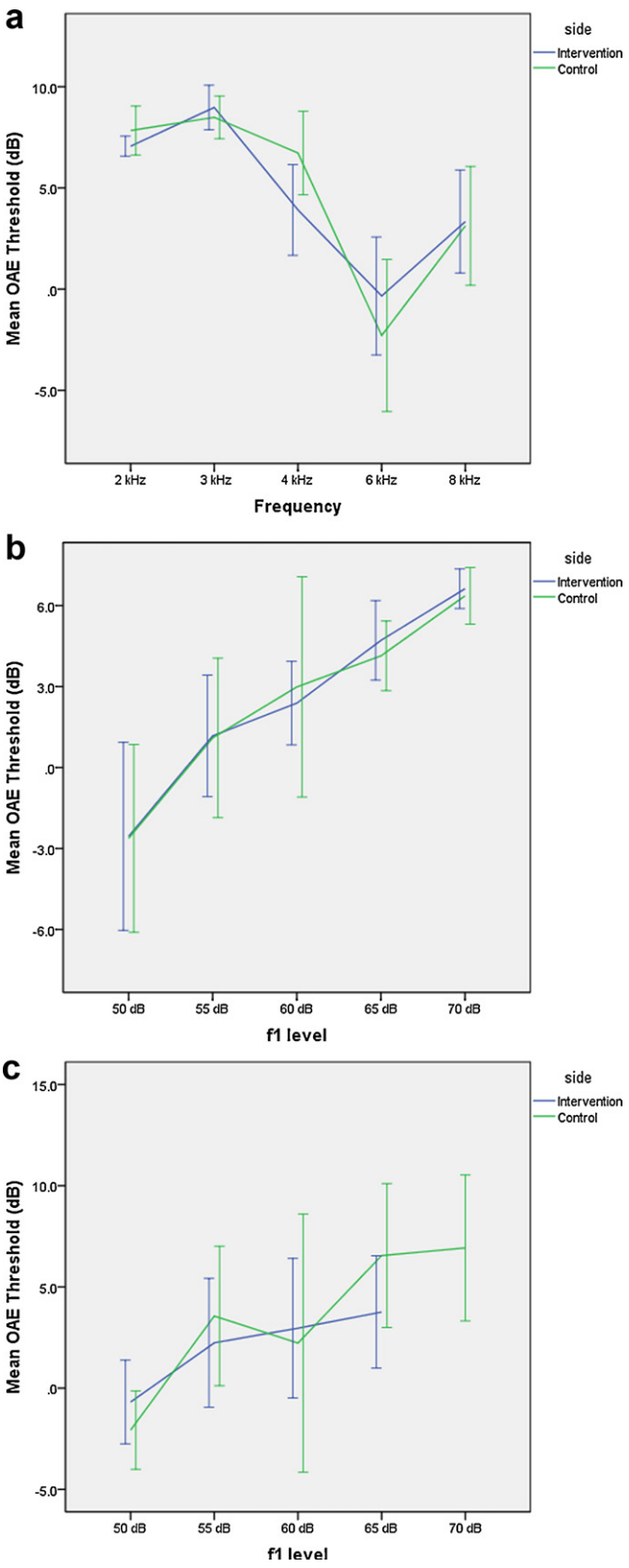

Fig. 4. (a) DP-gram in noise induced deaf animals after stem cell injection. (b) DP-IO for 6 kHz in deaf animals after stem cell injection. (c) DP-IO for 8 kHz in deaf animals after stem cell injection.

see any physiologic function either [13]. They repeated their work in vitro to find the best way to increase the possibility of the differentiation of the cells [8]. They found that the problem was in differentiation stage. These findings show that in future we should do some work to conduct BMSC differentiation to the hair cells.

Other explanation for our results may be poor survival of BMSCs in the cochlea. There are studies showing that these cells survive in the cochlea and the problem is somewhere else [14]. However, in future tracing survived BMSCs and differentiation process will help to find out the problem in stem cell transplantation in cochlea [7].

We tried to consider any possible confounding factor that may affect our results, and by injecting fluorescent dye in the cochlea, and performing sham surgery on deaf and normal hearing animals, we excluded any technical errors or confounding effect of surgery on the hearing results.

5. Conclusion

Previous studies have shown that stem cells have the ability to make cochlea and other inner ear structures, and theoretically they can replace the injured elements. However, our results show that these implanted cells with normal histologic structures have no physiologic function and hearing rehabilitation. Further studies by monitoring the survival of these cells with histologic and appropriate biomarkers will help to investigate differentiation process of these cells.

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