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Role of Endogenous Bone Marrow Stem Cells Mobilization in Repair of damaged inner ear in rats

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Abstract

Objective: The present work assessed the possibility of mobilizing endogenous bone marrow derived stem cells (SCs) in rats using granulocyte colony stimulating factor (G-CSF) to induce regeneration and repair to experimentally damaged inner ear hair cells by Amikacin injection.

Material & Methods: The study included thirty adult Sprague Dawley male rats. Experimental induction of inner ear damage was done by repeated intratympanic injection of amikacin sulfate. Mobilization of bone marrow SCs was provoked by subcutaneous injection of GCSF. Cochlear integrity, induction of hearing loss and functional recovery of sensory hearing loss were assessed using Distortion Product Optoacoustic Emission (DPOAEs). The morphological alteration and recovery of the organ of Corti was assessed histologically using the light and scanning electron microscopes.

Results: After six month duration, there was improvement in 50% of the sensorineural DPOAE results. Functional recovery coincided with the repair of structural components of organ of Corti.

Conclusion: SCs mobilization by G-CSF is a promising alternative method for replacement therapy in sensorineural hearing loss.

Introduction: The inner ear is composed of two main parts. auditory portion includes the cochlea, involved in hearing, and the vestibular system involved in balance.(1, 2, 3) Finding ways to cure deafness represent a major scientific and clinical breakthrough. Recently, stem cells from inner ear of adult mice have been identified. (4) These adult stem cells are found in the utricle of vestibular region of the inner ear. The discovery of such cells is a first step towards a promising line of treatment in restoring hearing and balance function. (4, 5) Stem cells are unspecialized cells that have two defining properties: the ability to differentiate into other cells and the ability to self-regenerate. (6) Adult stem cells are stem cells that can be derived from different parts of the body and, depending on where they are from, have different properties. They exist in several different tissues including bone marrow, blood and brain. Some studies have suggested that adult stem cells are very versatile and can develop into different cell types. (5 , 6)

Mobilization of endogenous stem cells provides an alternative way of replacing damaged inner ear hair cells, and correcting hearing loss. Mobilizing host stem cells is less cumbersome than transplantation in that it avoids the logistical complexity associated with the use of embryonic as well as non embryonic stem cells, including supply, surgical trauma, and possibilities of graft rejection, uncontrolled graft cell proliferation and tumor formation.

Aim: The aim of this work was to assess the use of G-CSF (Granulocyte Colony Stimulating Factor) to mobilize Bone Marrow Stem Cells to reach the inner ear of rats and its ability to repair damaged inner ear by Amikacin sulfate.

Materials & Methods: Thirty adult male Sprague-Dawley rats were supplied by Veterinary Department of Medical Research Institute, University of Alexandria. They were randomly divided into two groups: Group A 15 rats which were histologically examined as a negative control for the other injected group. Group B 15 rats which were subjected to the following:

1) Intra-tympanic injection under Microscopic magnification with Amikacin sulfate at starting dose of 5mg/kg bilaterally in 10 rats and unilaterally in 5 rats.

2) DPOAE of all injected rats (Group B) was done to assess the hearing function 3weeks after first Amikacin injection. Intra-Tympanic Amikacin injection was repeated until we had negative DPOAE of all injected rats (Group B).

3) Subcutaneous injection of GCSF (Granulocyte Colony Stimulating Factor) at a dose of 200 microgram/kg/day for 5 successive days for 10 rats of the 15 rats injected with Amikacin (Group B).

4) One month after GCSF treatment all rats were examined with DPOAEs to assess the hearing function and the same was repeated every month till 6 month.

5) Dissection of 2 rats was done every month one of each group by Light microscopy using routine hematoxylin and eosin (H&E) stain and also by Scanning electron microscopic examination of specimens (SEM).

Results: Table (1) & (2) illustrates statistical analysis of 6 months follow up DPOAEs assessment according to number of experimented rats and ears respectively. they shows that 50% of the rats (42% of numbers of ears) with amikacin sulfate induced hearing loss revealed statistically significant recovery of sensorineural hearing function six months after treatment with GCSF. ($p \leq 0.046$)

	M1		M2		M3		M4		M5		M6	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
-ve	8	100.0	8	100.0	6	75.0	5	62.5	4	50.0	4	50.0
+ve	0	0.0	0	0.0	2	25.0	3	37.5	4	50.0	4	50.0
P			1.000		0.157		0.083		0.046*		0.046*	

*: Statistically significant at $p \leq 0.05$

Table (1): 6 months follow up DPOAEs assessment according to Rats number

	M1		M2		M3		M4		M5		M6	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
-ve	12	100.0	12	100.0	10	83.3	9	75.0	7	58.3	7	58.3
+ve	0	0.0	0	0.0	2	16.7	3	25.0	5	41.7	5	41.7
p			1.000		0.157		0.083		0.025*		0.025*	

*: Statistically significant at $p \leq 0.05$

Table (2): 6 months follow up DPOAEs assessment according to Ears Number

Histological Results:

From the control group of rats we had an example of normal inner ear as seen in Light photomicrograph (Fig. 1) showing the normal structural components of the cochlear duct and the semicircular duct. And in (Fig. 2) we can see the cellular components of a normal Organ of Corti of a control inner ear.

From the group injected with Amikacin we had an example of light photomicrograph (Fig. 3) showing destruction of Organ of Corti and disappearance of the inner and outer supporting cells and infiltration of the cochlear cavity by red blood cells.

From the group of rats treated with GCSF the histological finding of an OAE positive rat recovering within variable durations after receiving GCSF therapeutic regimen can be demonstrated After 4 , 8 and 12 weeks (Fig.6a, 6b and 6c) the organ of Corti shows few outer hair cells. No other cellular components of the organ of Corti are identifiable.

After 24 weeks (Fig. 6d) a fully structured organ of Corti composed of intact rows of outer hair cells (OHC), outer pillar cells (OP) and an intact row of inner hair cells (IHC), inner pillar cells (IP). The outer (Oph) and inner (Iph) phalangeal cells border the inner tunnel (IT).

An example of a normal control rat organ of corti can be seen by scanning electron microscopy (fig. 7a) demonstrating normal hair cells (HC) with intact stereocilia projecting from their apices. and (Fig. 7b) showing surface view of the reticular lamina (RL) showing three parallel rows of intact stereocilia of the outer hair cells.

This can be compared to Scanning electron photomicrographs of the inner ear in an OAE negative (deaf) rat, 6 months after treatment with amikacin (Fig. 7c) showing the cochlear cavity is infiltrated by many red blood cells but no details of hair cells neither of its stereocilia can be identified. (Fig. 7d)

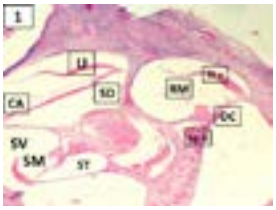

Fig. 1: Control Rat Inner Ear (Normal Rat)

Fig. 2: Control Rat Organ of Corti (Normal Rat)

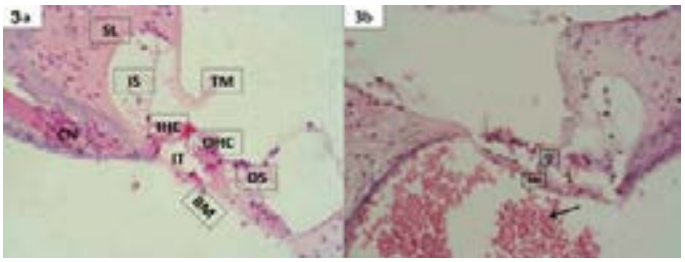

Fig. 3: Organ of Corti of Rat injected with Amikacin

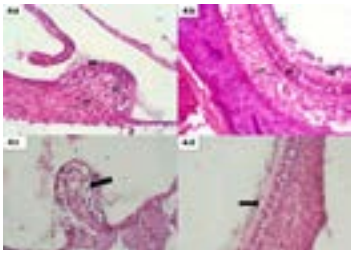

Fig. 4: Crista and Macula of normal rat then after Amikacin injection

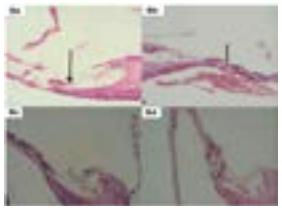

Fig 5: Organ of Corti of recovering rat after GCSF injection with variable durations 4, 8, 12 and 24 weeks

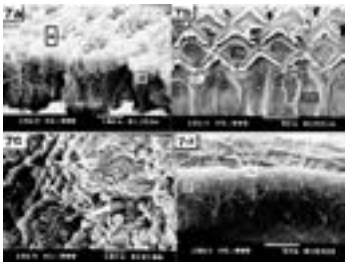

Fig. 7: Control Rat Organ of Corti by Electron Microscopy (SEM) and after Amikacin injection

Discussion:

The use of Stem Cells in the repair of damaged inner ear was first tried by Ito et al. in 2001 using neural stem cells. [9] And also in 2003 they studied the fate of neural stem cells grafted into injured inner ears of mice.[10]

In 2003 Li et al. have identified stem cells from the inner ear of adult mice. These adult stem cells are found in the utricle of the vestibular region of the inner ear. [11] This discovery has opened the door for many other researchers to explore the utilization of stem cells in the inner ear repair and restoration of hearing function.

Heller et al. in 2003 have studied the generation of hair cells by stepwise differentiation of embryonic stem cells. [12] And Parker et al. in 2004 studied the potential use of stem cells for cochlear repair. [13] Also many other stem cell studies have done in the next few years between 2003 and 2010 but most of these studies concentrated on the transplantation of stem cells into damaged inner ear or in vitro studies for generating hair cells from stem cells of different origins. [14,15]

At the beginning of the study we had to make sure of the hearing ability of all rats because some genetic malformations may exist in rats similar to human which is not uncommon, so we used a portable Oto-acoustic emission (OAE) as a screening tool for hearing function. We used aminoglycosides (Amikacin sulfate) to cause cochlear damage of the rat inner ear because it has a more cochlear side effect. And injected via Intra-Tympanic approach under microscopic magnification.

Amikacin injection was repeated until we had negative OAEs of all injected rats and this was reached in all rats after 9 weeks of the first Amikacin injection. We used a 5 day therapeutic regimen of subcutaneous injection of GCSF and the dose according to previous publications in literature in human is 5-10 Microgram/Kg/day but in rats it is 100-200 Microgram/Kg/day.[8] One month after GCSF treatment the rats were examined with DPOAEs to assess any improvement in the hearing function and the same was done every month till 6 month. After 6 month follow up we had 4 rats (5 ears) which were negative OAEs became positive OAEs showing clinical improvement in hearing function.

The group which was bilaterally injected with Amikacin we had 2 rats (3 ears) improved after GCSF treatment after a total follow up period of 6 month while in the group which was unilaterally injected with Amikacin we had improvement in 2 rats (2 ears). After treating Amikacin injected rats with GCSF, histological examination was done on variable durations and showed improvement in arrangment of hair cells till we reached complete restoration of Organ of Corti after 6 month and this was consistant with the clinical results of OAEs. While histological examination of the positive control rats (which were injected with Amikacin and not treated with GCSF) showed no improvement and this was also consistant with the clinical results.

Conclusions:

The use of Stem cells in hearing loss correction is a widely growing field in the last few years. Stem cells mobilization is an alternative method for transplantation in replacing damaged inner ear hair cells, and correcting hearing loss that it avoids the logistical complexity associated with the use of embryonic as well as non embryonic stem cells, including supply, surgical trauma, and possibilities of graft rejection, uncontrolled graft cell proliferation and tumor formation. Aminoglycosides have an established ototoxic side effect, Amikacin has a more cochlear effect than vestibular one especially when injected intra-tympanic to avoid its systemic effects. And so it is considered a very good tool for producing an animal model for inner ear damage and hearing loss.

G-CSF is one of many other well known inducers for stem cell mobilization from the bone marrow, and also it was approved from the FDA to be used in human in many other fields like in myocardial infarction and cerebrovascular stroke. G-CSF can be used successfully in damaged inner ear hair cells to induce Stem cells mobilization from the bone marrow to home and reach the damaged organ which is the inner ear in our case and it can result in some degree of hair cell regeneration and hearing improvement. Hair cell regeneration can be assessed audiologically by DPOAEs and histologically by Microscopic examination of inner ear Organ of Corti.

Future studies should utilize more animal models and to be followed up over an extended period of time and we also recommend the use of ABR to assess the hearing function quantitavely better than the OAEs alone. Also a measure of stem cell mobilization is recommended to be used as blood analysis for stem cells. and lastly detection of stem cells in the inner ear by immunohistochemistry is recommended to be used.

Disclosures:

There are no disclosures to declare.

References:

1. Gulya AJ, Anson BJ. Anatomy of the ear and temporal bone. In: Shambaugh GE, Glasscock ME III, editors. Surgery of the ear 5th ed. B.C. Decker Inc.; 2003. p. 35–57

2. Michaels L, Hellquist H. Ear, nose and throat histopathology. 2nd ed. Springer, London; 2001

3. Lim DJ. Functional structure of organ of Corti. Hearing Research 1986; 22: 117-46

4. Sell S. Stem cells. Stem Cell Handbook ed. by Sell S. 2004; 1-18

5. Blau HM, Brazelton TR, Weimann JM. The evolving concept of a stem cell: entity or function? Cell 2001; 105:829-841

6. Nagler A, Bellehsen L, Schaffer F. Stem Cells: Clinical Applications. Adkinson: Middleton's Allergy, 7th ed. 2008; 259-68

7. Parker MA, Cotanche DA. Neural Stem Cells injected into the sound-damaged cochlea migrate throughout the cochlea and express markers of Hair Cells, Supporting Cells, and Spiral Ganglion Cells. Hear Res 2007; 232(1-2): 29–43

8. Demetri GD, Griffin JD. Granulocyte colony-stimulating factor and its receptor. Blood 1991; 78:2791-808

9. Ito J, Kojima K, Kawaguchi S. Survival of neural stem cells in the cochlea. Acta Otolaryngology 2001; 121:140–42

10. Ito J, Iguchi F , Nakagawa T, Tateya I, Endo T, Kim TS, , et al. Fate of neural stem cells grafted into injured inner ears of mice. Neuroreport 2003; 14: 1677-81

11. Li H, Liu H, Heller S. Pluripotent stem cells from the adult mouse inner ear. Nat Med 2003; 9:1293–9

12. Heller S, Li H, Roblin G, Liu H. Generation of hair cells by stepwise differentiation of embryonic stem cells. Proc Natl Acad Sci USA. 2003; 100(23):13495–500

13. Parker MA, Cotanche DA. The potential use of stem cells for cochlear repair. Audol Neurootol 2004; 9:72–80

14. Martinez-Monedero R, Oshima K, Heller S, Edge ASB. The potential role of endogenous stem cells in regeneration of the inner ear. Hear Res 2007; 227(1-2): 48–52

15. Li H, Corrales CE, Edge A, Heller S. Stem cells as therapy for hearing loss. Trends Mol. Med 2004; 10:309–15