Egyptian Journal of Ear, Nose, Throat and Allied Sciences (2015) **16**, 55–67

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Egyptian Journal of Ear, Nose, Throat and Allied Sciences

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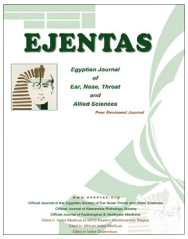

ORIGINAL ARTICLE

Endogenous bone marrow stem cell mobilization in rats: Its potential role in homing and repair of damaged inner ear

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Received 20 November 2012; accepted 26 January 2013
Available online 16 March 2013

KEYWORDS

Stem cells;
Mobilization;
Inner ear;
Rats;
Hearing;
Amikacin

Abstract The stem cells are widely used in the last few years in different fields of medicine, either by external transplantation or endogenous mobilization, most of these studies are still experimental on animals; few were tried on humans as in the spinal cord injury or myocardial infarction. As regards its use in the inner ear, stem cell transplantation was examined in many previous studies, while the mobilization idea is a new method to be experimented in inner ear hair cell regeneration.

The aim of this work was to assess the use of GCSF to induce bone marrow SC mobilization to home and repair the damaged inner ear hair cells in rats after the damage had been inflicted by Amikacin injection.

First we had to develop an animal model for damaged inner ear, so we used the well known ototoxic effect of Amikacin intra-tympanic injection till we reached complete loss of hearing function. And this can be assessed by DPOAEs. Now we have an animal model of damaged inner ear hair cells and mobilization of bone marrow SCs was induced by subcutaneous injection of G-CSF for 5 days.

Clinical assessment was done using DPOAEs before and after G-CSF injection also histological assessment of the inner ear was done before and after G-CSF by two methods H&E staining and Scanning Electron Microscopy.

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Peer review under responsibility of Egyptian Society of Ear, Nose, Throat and Allied Sciences.

http://dx.doi.org/10.1016/j.ejenta.2013.01.003

The results of histological examination of the specimens showed some promising changes that may be responsible for the improvement of hearing function in some rats that was detected by OAEs.

1. Introduction

The inner ear is composed of two main parts. The auditory portion includes the cochlea, which is involved in hearing, and the vestibular system which is involved in balance. Our ability to hear and our sense of balance are critically dependent on specialized sensory receptors called hair cells, these cells have structures called stereocilia, which sense sounds by bending back and forth, converting mechanical vibrations into electrical, or neural signals that are then passed to the brain through the auditory nerve.[1,2]

Auditory hair cells, like a microphone, are designed to convert sounds into neural activity. Vestibular hair cells, those located in structures called the semicircular canals; detect head rotation, whereas other vestibular hair cells, located in structures called the utricle and saccule, sense head orientation with respect to gravity.[2,3]

Finding ways to cure deafness represent a major scientific and clinical breakthrough.[1] Recently, stem cells from the inner ear of adult mice have been identified.[4] These adult stem cells are found in the utricle of the vestibular region of the inner ear. They have the characteristic features of stem cells such as the capacity for self renewal and expansion (they divide and multiply). They form spheres, which begin to differentiate into new cell types termed as progenitor cells. Some progenitor cells differentiate into cells that express proteins and genes present in the developing inner ear and nervous system. Under appropriate conditions, some cells differentiate into cells resembling hair cells, which have stereocilia; hair bundles protruding from their surface and express specific hair cell marker proteins. The discovery of such cells is a first step toward a promising line of treatment in restoring hearing and balance function.[5,6]

Stem cells are unspecialized cells that have two defining properties: the ability to differentiate into other cells and the ability to self-regenerate. The ability to differentiate is the potential to develop into other cell types. A totipotent stem cell (e.g. fertilized egg) can develop into all cell types including the embryonic membranes. A pluripotent stem cell can develop into cells from all three germinal layers (e.g. cells from the inner cell mass). Other cells can be oligopotent, bipotent or unipotent depending on their ability to develop into few, two or one other cell type(s). Self-regeneration is the ability of stem cells to divide and produce more stem cells.[6,7]

There are two main types of stem cells: embryonic and adult stem cells. Embryonic stem cells are derived from embryos. Typically 4 or 5 days old and consist of a hollow microscopic collection of cells called the blastocyst. Unlike adult stem cells, embryonic stem cells can become all cell types because they are pluripotent.[8–11]

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 the bone marrow, the blood and the brain. Some studies have suggested that adult stem cells are very versatile and can develop into many different cell types.[12,13]

Hematopoietic stem cells are adult stem cells found mainly in the bone marrow and they provide the blood cells required for daily blood turnover and for fighting infections. Compared to adult stem cells from other tissues, hematopoietic stem cells are easy to obtain, as they can be either aspirated directly out of the bone marrow or stimulated to move into the peripheral blood stream, where they can be collected easily .[14–19]

More recently, their use in treatment of breast cancer and coronary artery diseases has also been explored.[20] The potential for hematopoietic stem cells to produce cell types other than blood cells has become the subject of intense scientific controversy, and it is still not clear whether they could be used on a clinical scale to restore tissues and organs other than blood and the immune system.[21,22]

Mesenchymal stem cells are another well-characterized population of adult stem cells. These cells, also found in the bone marrow, can form a variety of cells in the laboratory, including fat cells, cartilage, bone, tendon and ligaments, muscles cells, skin cells and even nerve cells. Unlike most other human adult stem cells, mesenchymal stem cells can be obtained in quantities appropriate for clinical applications, making them good candidates for use in tissue repair.[16,23]

From a scientific perspective, scientists will first need to identify compounds and conditions that can increase the growth of stem cells and promote their differentiation into hair cells or supporting cells.[24] From a clinical perspective, surgical and technical procedures need to be developed to successfully transplant stem cells into the inner ear. The critical question to be answered is whether transplanted stem cells can migrate to the correct location, differentiate into hair cells and restore hearing or balance.[25,26]

Mobilization of endogenous stem cells provides an alternative way of replacing damaged inner ear hair cells, and correcting hearing loss.[19,22] 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.

2. Aim

The aim of this work is 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 after injecting Amikacin sulfate into the middle ear of rats.

3. Materials

3.1. Animals

- The study was carried out on 30 adult male rats of Sprague–Dawley strain from the Medical Research Institute experimental laboratory of the Veterinary Department of Alexandria Medical School. The study was approved by the medical ethics committee of Alexandria Faculty of Medicine.

3.2. Drugs

- The procedure was done under general sedative anesthesia using IM injection of Ketamine hydrochloride (50 μg/kg).
- Amikacin sulfate at starting dose of (5 μg/kg) was injected intra-tympanic under microscopic magnification and repeated until we reached complete cochlear damage.
- G-CSF (Granulocyte Colony Stimulating Factor) at a dose of 200 μg/kg/day was injected subcutaneously for 5 days.

3.3. Instruments

- The intra-tympanic injection will be done under microscopic magnification (Carl Zeiss laboratory microscope).
- Electrophysiological studies using the DPOAE (Distortion product Otoacoustic Emission) will be used to assess cochlear integrity before the start of procedure and after injection of Amikacin sulfate to adjust the optimal dose for complete cochlear damage. Then serial measurement will be done after injecting G-CSF to assess any improvement of cochlear function.

4. Methods

The study has been conducted on 30 adult male rats of Sprague–Dawley strain from the Medical Research Institute experimental laboratory of the Veterinary Department.

All rats were examined under microscopic magnification for the tympanic membrane to exclude any ear abnormalities and then subjected to DPOAEs to assess their cochlear integrity (to ensure normal hearing before start of the trial). And the rats which were deaf were excluded from the study.

Rats were randomly divided into two groups:

Group A consists of 15 rats which were dissected and the inner ear was histologically examined as a negative control for the other injected group.

Group B consists of 15 rats which were subjected to the following:

(1) Intra-tympanic injection under microscopic magnification with Amikacin sulfate at starting dose of 5 μg/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 after first Amikacin injection.
(3) Intra-tympanic Amikacin injection was repeated until we had negative DPOAE of all injected rats (Group B) at 3, 6 and 9 weeks after first Amikacin injection.
(4) Subcutaneous injection of GCSF (Granulocyte Colony Stimulating Factor) at a dose of 200 μg/kg/day for 5 successive days for 10 rats of the 15 rats injected with Amikacin (Group B).

The other 5 rats were kept untreated with GCSF as positive control (hearing damaged by Amikacin but not treated with GCSF).

(5) One month after GCSF treatment all rats were examined with DPOAEs to assess any improvement in the hearing function and the same was repeated every month till 6 months.
(6) Dissection of 2 rats was done every month one of each group and the inner ear was histologically examined.
(7) After the follow up period of 6 months, all the remaining rats were dissected and the inner ear was histologically examined.

Dissection was done as following:

1. Each rat was euthanized and decapitated and the skin removed.
2. The head was cut into two halves by making an incision at the midline.
3. The brain and brainstem were removed to expose the temporal bone.
4. The petrous temporal part of the skull was trimmed and sectioned longitudinally to expose the internal structures of the inner ear.
5. During dissection of specimens we kept them wet with 4% formaldehyde – 1% gluteraldehyde (4F-1G).
6. Specimens were labeled with rat numbers and sent for histological examination.

Histological examination of the specimens was done as following:

(1) Light microscopic examination of paraffin sections using routine hematoxylin and eosin (H&E) stain.[17]
(2) Scanning electron microscopic examination of specimens (SEM):
   1. Fixation of specimen in 3% buffered gluteraldehyde pH 7.3 for 2 h at 4 c followed by 4% formaldehyde – 1% gluteraldehyde (4F-1G) for 48 h.
   2. Washing in phosphate buffer for 15 min.
   3. Post fixation in freshly prepared 1% osmium tetraoxide in phosphate buffer at 4 c for 2 h with agitation.
   4. Dehydration in ascending grades of acetone for 15 min.
   5. Complete dehydration in isoamyl acetate for 15 min.
   6. Critical point drying by liquid carbon dioxide at 40 c at 1500 psi pressure.
   7. Mounting on copper stubs and vacuum evaporation with gold palladium film layering.
   8. Examination by Joel scanning electron microscope 5–25 kV, Electron Microscopy Unit, Faculty of Science, University of Alexandria.[18]

5. Results

The study has been conducted on 30 adult male rats of Sprague–Dawley strain. They were randomly divided into two groups: Group A consists of 15 rats not injected with Amikacin (−ve Control). Group B consists of 15 rats which were all injected intra-tympanic under Microscopic magnification with Amikacin sulfate at a starting dose of 5 μg/kg, 5 rats were injected unilaterally and 10 rats were injected bilaterally (see Table 1).

After 3 weeks of first Amikacin injection all injected rats (Group B) were examined by DPOAE signals.

Table 1 Distribution of rats at the start of the study.

	No.	%
Control rats (Group A)	15	50.0
Bilaterally injected rats	10	33.0
Unilaterally injected rats	5	17.0

six rats of the 15 rats became deaf after 3 weeks of Amikacin injection, while the other 9 rats had a positive OAE and still preserve residual hearing function. So intra-tympanic Amikacin injection was repeated and waited another 3 weeks.

After 6 weeks of first Amikacin injection all injected rats (Group B) were examined again by DPOAEs.

Another 6 rats of the 15 rats became deaf 6 weeks after first Amikacin injection, while only 3 rats still had positive OAEs and preserve residual hearing function. So intra-tympanic Amikacin injection was repeated and waited another 3 weeks.

After 9 weeks of first Amikacin injection all injected rats (Group B) were examined again by DPOAEs and they were all negative (complete loss of hearing function).

Subcutaneous injection of GCSF (Granulocyte Colony Stimulating Factor) at a dose of 200 μg/kg/day for 5 successive days was administered as shown in Table 2.

The other 5 rats of group B were bilaterally injected with Amikacin and not treated with GCSF (Positive Control).

After one month of GCSF treatment all rats were examined with DPOAEs to assess any improvement in the hearing function and the same was done every month till 6 months.

Table 2 GCSF treatment.

5 rats of group B	Bilateral ear deafness induced by Amikacin and treated with GCSF
5 rats of group B	Unilateral ear deafness by Amikacin and treated with GCSF

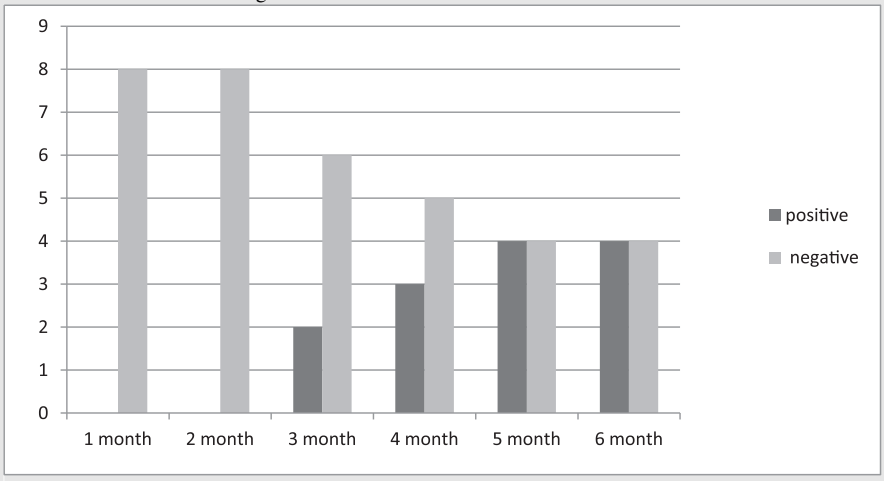
N.B: one rat was dead from each group so we had 4 rats with unilateral ear deafness and 4 rats with bilateral ear deafness and treated with GCSF.

Table 3 6 months follow up on DPOAEs assessment according to rat numbers.

	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*						

P: *p* value for McNemar test.

6 months follow up DPOAEs assessment according to Rats number.



6 months follow up DPOAEs assessment according to Rats number.

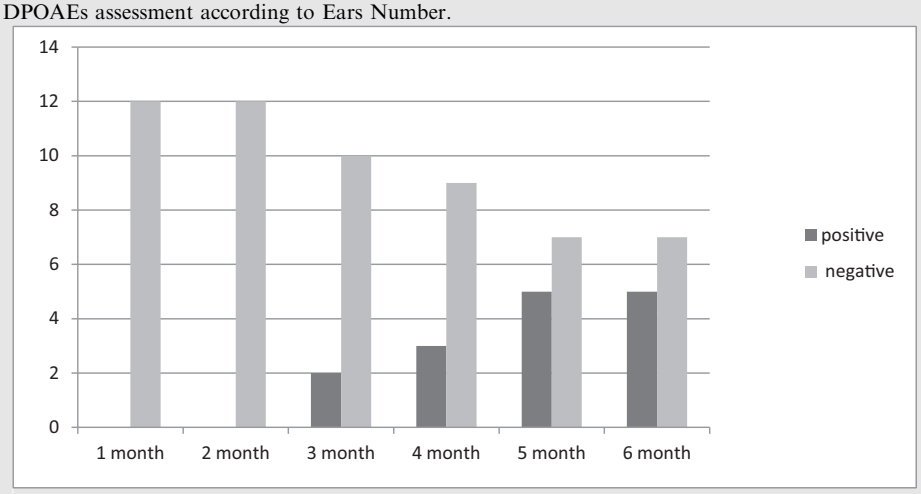
* Statistically significant at $p \leqslant 0.05$.

Table 4 6 months follow up on DPOAEs assessment according to ear numbers.

	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*						

P: *p* value for McNemar test.

6 months follow up DPOAEs assessment according to Ears Number.



6 months follow up DPOAEs assessment according to Ears Number.

* Statistically significant at $p \leqslant 0.05$.

5.1. Audiological results

In the first 2 months after GCSF was given no improvement could be detected in hearing with Oto-Acoustic Emissions.

In the third month we had 2 rats (2 ears one from the unilaterally injected group and the other ear from the bilaterally injected group) which were negative OAEs became positive OAEs.

In the fourth month we had 3 rats (3 ears one from the unilaterally injected group and two ears from the bilaterally injected group) which were negative OAEs became positive OAEs.

In the fifth month we had 4 rats (5 ears two from the unilaterally injected group and 3 ears from the bilaterally injected group) which were negative OAEs became positive OAEs.

In the last month we had 4 rats (5 ears two from the unilaterally injected group and 3 ears from the bilaterally injected group) which were negative OAEs became positive OAEs (see Tables 3 and 4).

As regards rat numbers we noticed that in the first 2 months after GCSF treatment no improvement occurred ($P = 1.000$) and in the third month there was improvement in 2 rats ($P = 0.157$) and in the fourth month there was improvement in 3 rats ($P = 0.083$) while in the fifth and sixth month there was improvement in 4 rats out of 8 rats injected with Amikacin ($P = 0.046$).

As regards ear numbers we noticed that in the first 2 months we had no improvement ($P = 1.000$) and in the third month there was improvement in 2 ears ($P = 0.157$) and in the fourth month there was improvement in 3 ears ($P = 0.083$) while in the fifth and sixth there was improvement in 5 ears out of 12 ears injected with Amikacin ($P = 0.025$).

5.2. Histological results

From the control group of rats we had an example of the normal inner ear as seen in light photomicrograph (Fig. 1) showing the normal structural components of the cochlear duct and the semicircular duct.

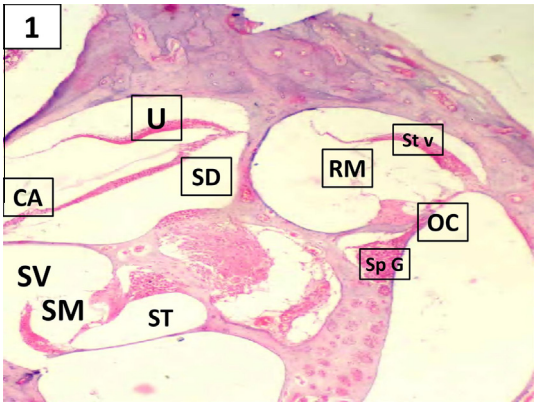


Figure 1 Control rat inner ear (normal rat). Light photomicrographs of control rat inner ear cavity demonstrating the cochlear cavity divided by the Reissener's membrane (RM) into scala media (SM) and scala vestibuli (SV). The organ of Corti (OC) intervenes between the scala media and the scala tympani (ST). It is boundered laterally by the stria vascularis (St V) and medially by the spiral ganglion (SG). The semicircular duct (SD) shows the macula utriculi (U) and the crista ampullaris (CA) (H&E stain. Mic. Mag. ×40).

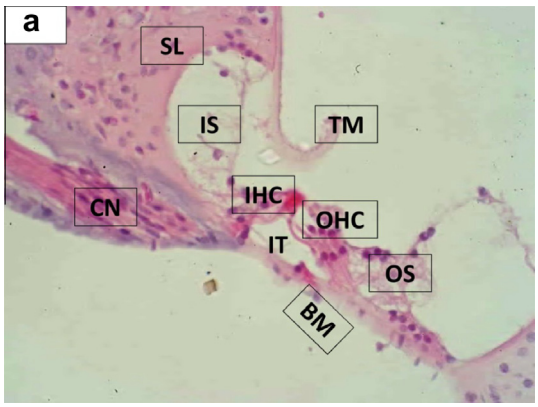


Figure 2a Control rat organ of Corti (normal rat). Light photomicrographs of control rat Organ of Corti showing: the main structural components including the spiral limbus (SL), tectorial membrane (TM), inner supporting cells (IS), inner hair cells (IHC), inner tunnel (IT), outer hair cells (OHC), outer supporting cells (OS), basilar membrane (BM) and the related cochlear nerve (CN) (H&E stain. Mic. Mag. ×400).

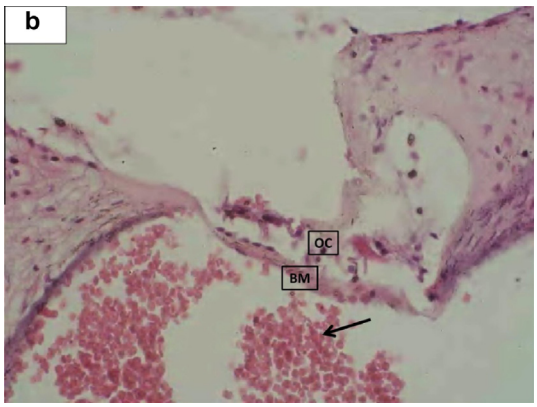


Figure 3 Organ of Corti of rat injected with Amikacin. An OAE negative rat (deaf), 6 months after administration of amikacin revealing destruction of the organ of Corti (OC), disappearance of the inner and outer supporting cells and infiltration of the cochlear cavity by red blood corpuscles (arrow). Note the intact basilar membrane (BM) (H&E stain. Mic. Mag. ×400).

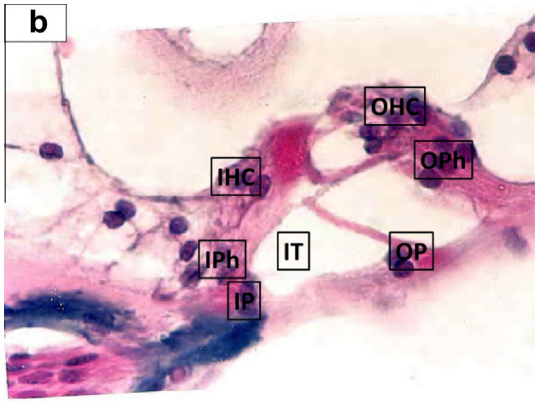


Figure 2b Higher magnification of the cellular components of control rat organ of Corti demonstrating a single row of inner hair cells (IHC) supported by the underlying inner phalangeal cells (IPhC), and several rows of outer hair cells (OHC) supported by the outer phalangeal cells (OPhC). The inner tunnel (IT) is bounded between the inner (IP) and outer pillar (OP) cells (H&E stain. Mic. Mag. ×1000).

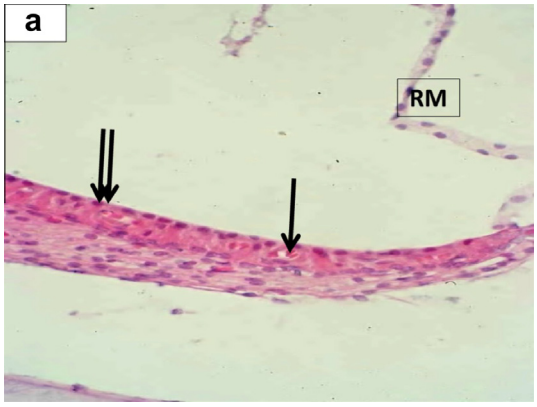


Figure 4a Control rat stria vascularis. Light photomicrograph of rat control stria vascularis shows an intact superficial layer of flat epithelial cells (double arrows) and normal multiple intraepithelial blood capillaries (single arrow) (H&E stain. Mic. Mag. ×400).

Also in Fig. 2a with higher magnifications (Mic. Mag. ×400) we can see the structures of normal Organ of Corti of a control rat inner ear.

With further higher magnifications (Mic. Mag. ×1000) (Fig. 2b) 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, disappearance of the inner and outer supporting cells and infiltration of the cochlear cavity by red blood cells.

The stria vascularis of a normal control rat can be seen in Fig. 4a with an intact superficial layer of flat epithelial cells and normal multiple intraepithelial blood capillaries. And this can be compared to the stria vascularis of an OAE negative rat (deaf) 6 months after amikacin treatment (Fig. 4b) revealing focal denudation of superficial epithelium with vacuolation and widening of stromal interstitial spaces.

The semicircular canal of a normal control rat inner ear (Fig. 5a) and with higher magnification we can see the detailed structure of normal crista ampullaris (Fig. 5b) and normal macula utriculi (Fig. 5c) and this can be compared to the crista ampullaris of an OAE negative rat (deaf) 6 month after amikacin treatment as seen in Fig. 6a which reveals vacuolation and widening of interstitial spaces between hair and supporting cells, but we noticed that many hair cells (HC) are still intact. And also the macula utriculi in Fig. 6b shows intact layers of hair and supporting cells and this indicates that the vestibular structures are still intact or mildly affected after Amikacin injection while its main effects were on the cochlear structures.

The spiral ganglion of a normal control rat inner ear that can be seen in Fig. 7a demonstrates a closely packed group of intact bipolar cells (BC) with normal eccentric nuclei and prominent nucleoli. And this can be compared to the spiral

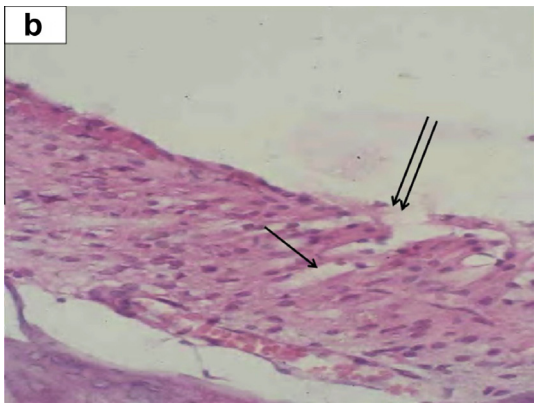


Figure 4b Stria vascularis of a rat injected with Amikacin. The stria vasularis from an OAE negative (deaf) rat 6 month after amikacin injection revealing focal denudation of superficial epithelium (double arrows) with vacuolation and widening of stromal interstitial spaces (single arrow) (H&E stain. Mic. Mag. ×400).

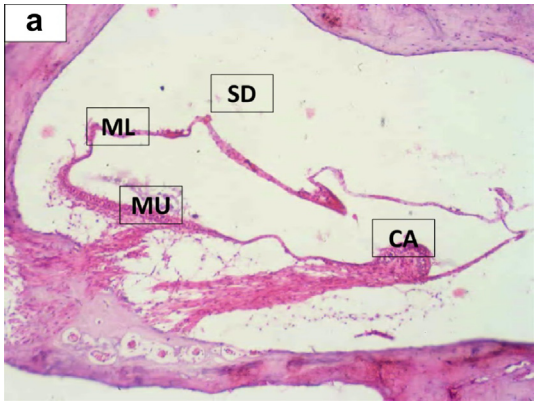


Figure 5a Control rat semicircular duct. Light photomicrograph of control rat semicircular duct (SD) lined by an intact membranous labyrinth (ML) showing normal focal thickening to form the macula utriculi (MU) and crista ampullaris (CA) (H&E stain. Mic. Mag. ×100).

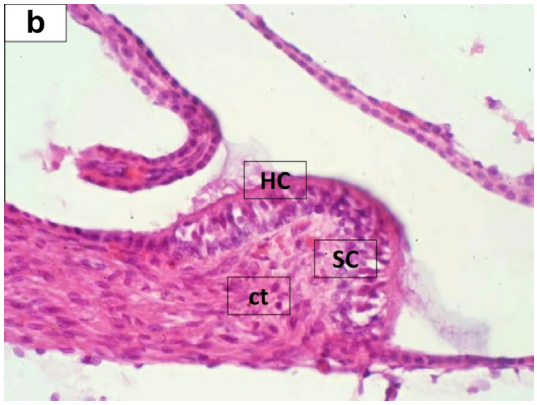


Figure 5b Control rat crista ampullaris. The crista ampullaris shows an intact superficial layer of hair cells (HC) supported by an underlying layer of supporting cells (SC) and connective tissue stroma (ct) (H&E stain. Mic. Mag. ×400).

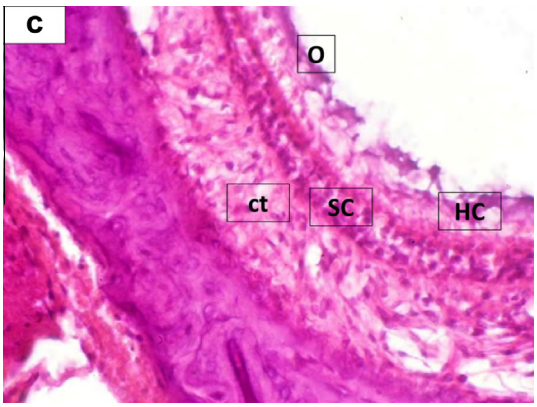


Figure 5c Control rat macula utriculi. The macula utriculi shows an intact superficial layer of hair cells (HC) and the overlying otoconia (O). It is supported by a deeper layer of supporting cells (SC) and connective tissue stroma (ct) (H&E stain. Mic. Mag. ×400).

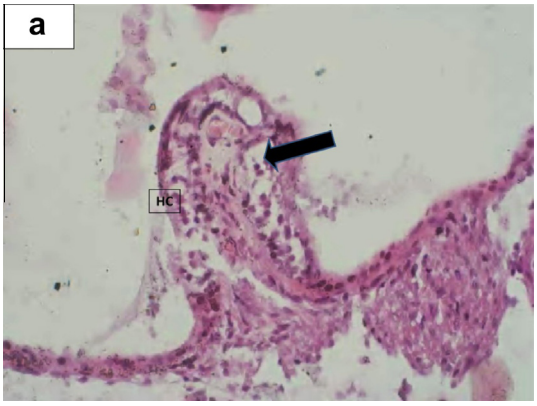


Figure 6a Crista ampullaris of a rat injected with Amikacin. Light photomicrograph of OAE negative rat, 6 months after injection with amikacin: the crista ampullaris reveals vacuolation and widening of interstitial spaces between hair and supporting cells (thick arrow). Note that many hair cells (HC) are still intact (H&E stain. Mic. Mag. ×400).

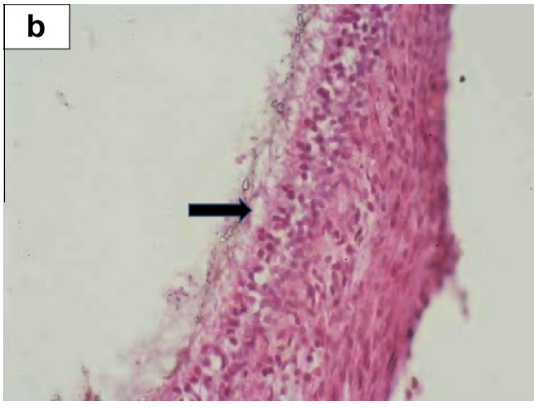


Figure 6b Macula utriculi of a rat injected with Amikacin. The macula utriculi shows intact layers of hair and supporting cells (arrow) (H&E stain. Mic. Mag. ×400).

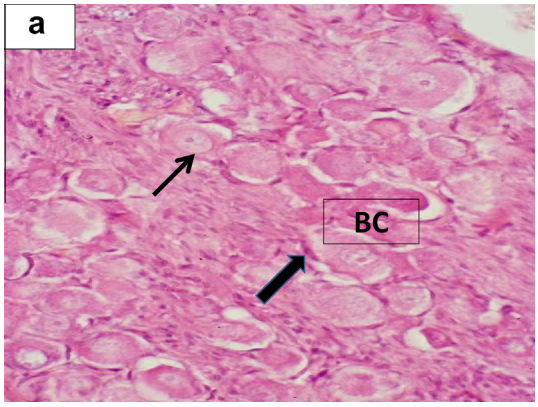


Figure 7a Control rat spiral ganglion. Light photomicrograph of rat control spiral ganglion demonstrates a closely packed group of intact bipolar cells (BC) with normal eccentric nuclei and prominent nucleoli (thin arrow). Each cell is surrounded by supporting satellite cells with flat nuclei (thick arrow) (H&E stain. Mic. Mag. ×400).

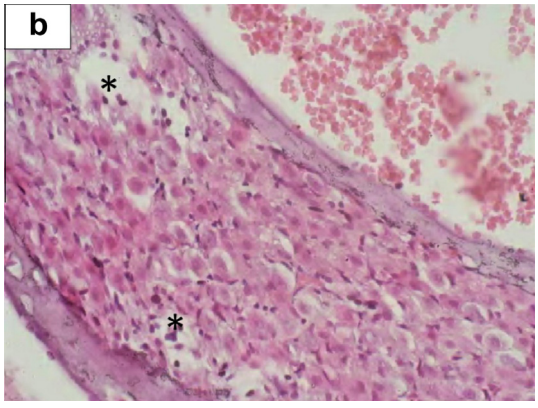


Figure 7b Spiral ganglion of a rat injected with Amikacin. The spiral ganglion from an OAE negative (deaf) rat, 6 months after injection with amikacin reveals loss of individual bipolar cells (*) leaving empty spaces behind (H&E stain. Mic Mag. ×400).

ganglion of an OAE negative rat (deaf) 6 months after amikacin treatment as seen in Fig. 7b which reveals the loss of individual bipolar cells leaving empty spaces behind.

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 as following:

After 4 weeks (Fig. 8a) the organ of Corti shows few outer hair cells. No other cellular components of the organ of Corti are identifiable.

After 8 weeks (Fig. 8b) the scala tympani reveals group of cells that are apparently organized into a primitive Organ of Corti, but morphological identification of individual hair and supporting cells is not yet clear.

After 12 weeks (Fig. 8c) the organ of Corti is formed of well structured rows of inner supporting cells (IS), inner hair cells (IH), outer hair cells (OH) and the intervening tunnels (T).

After 20 weeks (Fig. 8d) a fully structured Organ of Corti is noticed with identifiable detailed cellular and structural components.

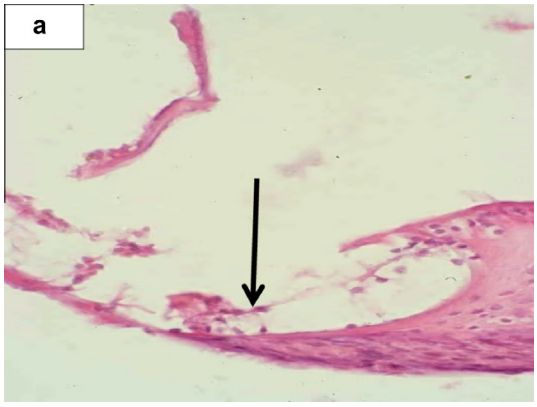


Figure 8a Organ of Corti of rat recovering after GCSF regimen after 4 weeks. Light photomicrographs of the organs of Corti of OAE positive rats recovering within variable durations from receiving GCSF therapeutic regimen: after 4 weeks, the organ of Corti shows few outer hair cells (arrow). No other cellular components of the organ of Corti are identifiable (H&E stain. Mic. Mag. ×400).

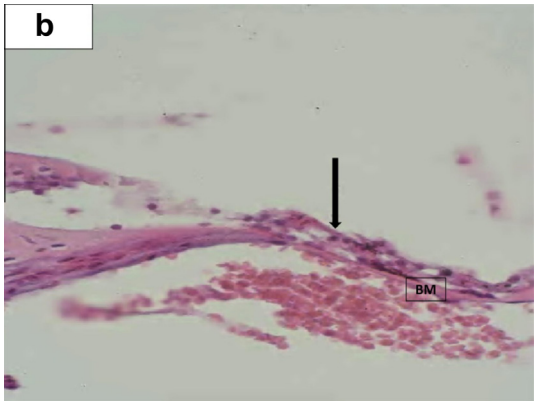


Figure 8b Organ of Corti of rat recovering after GCSF regimen after 8 weeks. After 8 weeks, the scala tympani reveals group of cells (arrow) that are apparently organized into a primitive Organ of Corti. Note that morphological identification of individual hair and supporting cells is not clear. SL = spiral limbus, BM = basilar membrane (H&E stain. Mic. Mag. ×400).

After 24 weeks (Fig. 9a) a fully structured organ of Corti is noticed composed of intact rows of outer hair cells (OHC), outer pillar cells (OP), an intact row of inner hair cells (IHC), and inner pillar cells (IP). The outer (Oph) and inner (Iph) phalangeal cells border the inner tunnel (IT). Also (Fig. 9b) the stria vascularis reveals a complete sheet of intact superficial epithelium with residual interstitial vacuolation in the supporting connective tissue stroma. Also intact macula utriculi (Fig. 9c) and intact crista ampullaris (Fig. 9d) is noticed. Also we can see intact spiral ganglion (Fig. 9e).

An example of a normal control rat organ of Corti can be seen by scanning electron microscopy (Fig. 10a) demonstrating normal hair cells (HC) with intact stereocilia projecting from their apices. And with higher magnifications (Fig. 10b) intact stereocilia of hair cells (Fig. 10c) surface view of the reticular

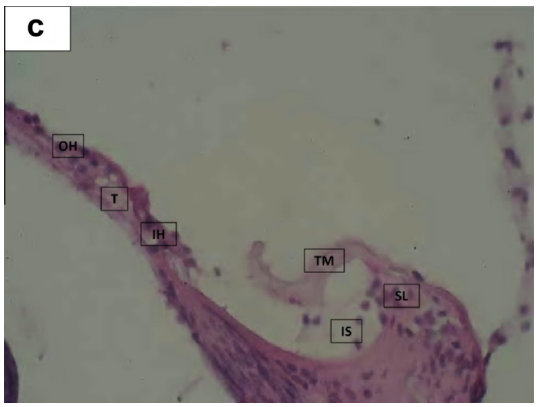


Figure 8c Organ of Corti of rat recovering after GCSF regimen after 12 weeks. After 12 weeks, the organ of Corti is formed of well structured rows of inner supporting cells (IS), inner hair cells (IH), outer hair cells (OH) and the intervening tunnels (T). SL = spiral limbus, TM = tectorial membrane (H&E stain. Mic. Mag. ×400).

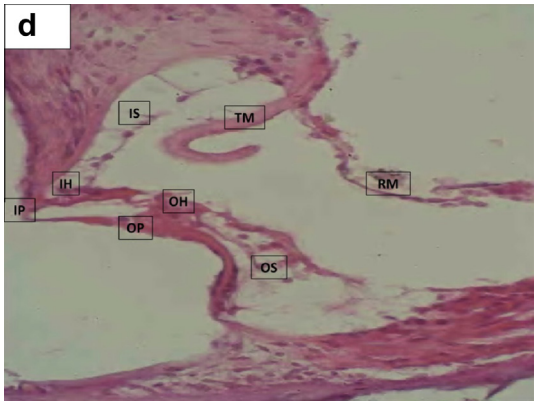


Figure 8d Organ of Corti of rat recovering after GCSF regimen after 20 weeks. After 20 weeks, a fully structured Organ of Corti is depicted with identifiable detailed cellular and structural components. (IS) inner supporting cells, (OS) outer supporting cells, (IH) inner hair cells, (OH) outer hair cells, (IP) inner pillar cells, (OP) outer pillar cells, (TM) tectorial membrane, (RM) Reissener's membrane (H&E stain. Mic. Mag. ×400).

lamina (RL) showing three parallel rows of intact stereocilia of the outer hair cells were observed.

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. 11a) showing the cochlear cavity is infiltrated by many red blood cells but no details of hair cells neither of its stereocilia can be identified. And with higher magnifications (Fig. 11b) the surface view of the reticular lamina (RL) of the organ of Corti showing parallel rows of empty indentations marking the lost outer hair cells was observed.

From the group of rats which have received GCSF we have an example of scanning electron photomicrograph of the cochlear cavity of an OAE positive rat, recovering after 24 weeks from receiving GCSF (Fig. 12) showing a group of intact hair cells (HC) supported by the underlying phalangeal cells (Ph C) and the hair cells show well formed apical stereocilia.

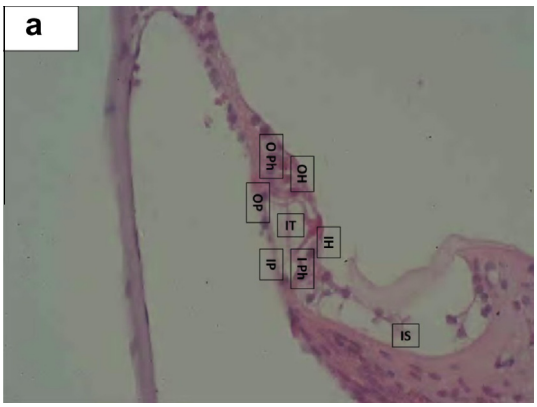


Figure 9a Organ of Corti of rat recovering after GCSF regimen after 24 weeks. Light photomicrographs of the inner ear from OAE positive rats recovering after 24 weeks from receiving GCSF therapeutic regimen: showing a normal, fully structured organ of Corti composed of intact rows of outer hair cells (OH), outer pillar cells (OP) and an intact row of inner hair cells (IH) supported by inner pillar cells (IP). The outer (Oph) and inner (Iph) phalangeal cells border the inner tunnel (IT). (IS) inner supporting cells (H&E stain. Mic Mag. ×400).

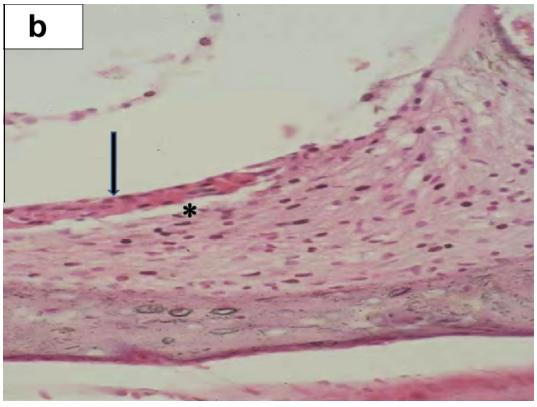


Figure 9b Stria vascularis of rat recovering after GCSF therapeutic regimen. The stria vascularis reveals a complete sheet of intact superficial epithelium (arrow) with residual interstitial vacuolation in the supporting connective tissue stroma (*) (H&E stain. Mic Mag. ×400).

6. 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.[19] And also in 2003 they studied the fate of neural stem cells grafted into injured inner ears of mice.[20]

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.[21] 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.[22] And

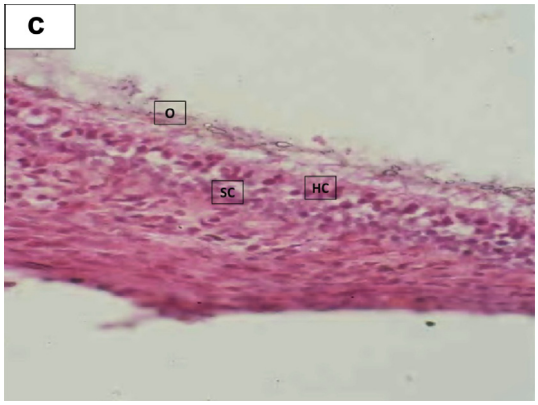


Figure 9c Macula utriculi of rat recovering after GCSF therapeutic regimen. The macula utriculi shows intact structural components: (HC) hair cells, (SC) supporting cells, (O) otoconia (H&E stain. Mic Mag. ×400).

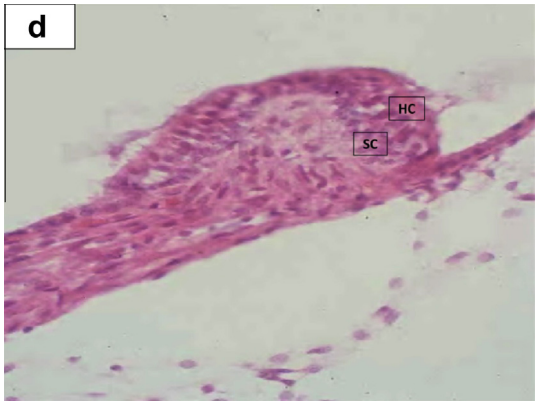


Figure 9d Crista ampullaris of rat recovering after GCSF therapeutic regimen. The crista ampullaris shows intact layers of hair cells (HC) and supporting cells (SC) (H&E stain. Mic Mag. ×400).

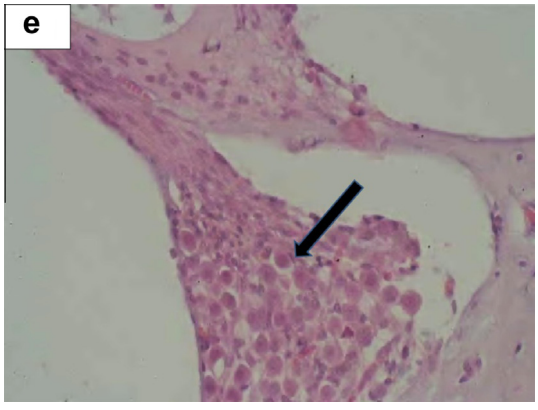


Figure 9e Spiral ganglion of rat recovering after GCSF therapeutic regimen. The spiral ganglion (arrow) is composed of intact bipolar cells with apparently average cell density (H&E stain. Mic Mag. ×100).

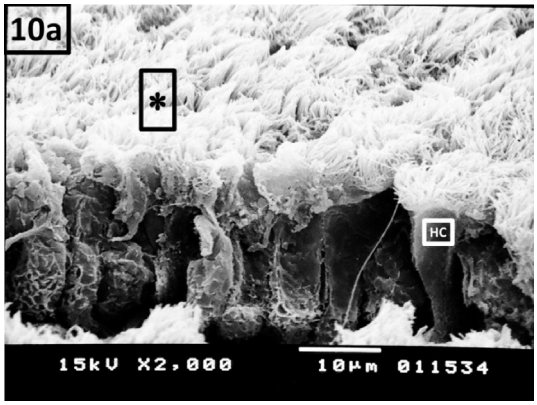


Figure 10a Control rat organ of Corti by scanning electron microscopy (SEM). Scanning electron photomicrograph of control rat organ of Corti demonstrating: normal hair cells (HC) with intact stereocilia (*) projecting from their apices (Mic. Mag. ×2000).

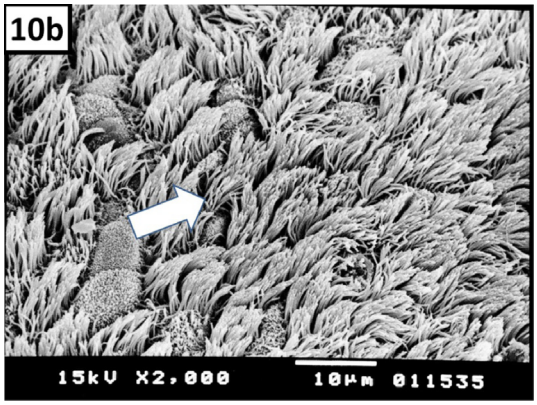


Figure 10b Control rat organ of Corti: surface view (SEM). Intact stereocilia of hair cells (arrow) (Mic. Mag. ×2000).

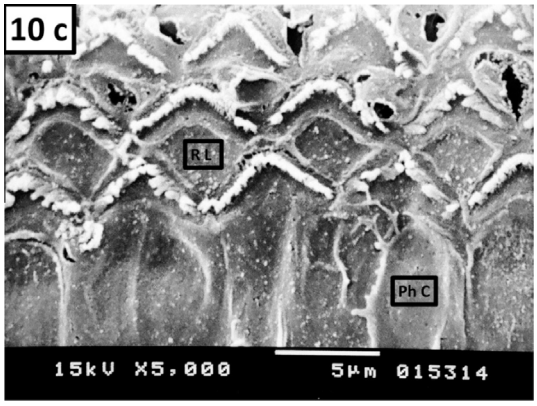


Figure 10c Surface view of the reticular lamina (RL) showing three parallel rows of intact stereocilia of the outer hair cells (Ph C) bodies of supporting outer phalangeal cells (Mic. Mag. ×5000).

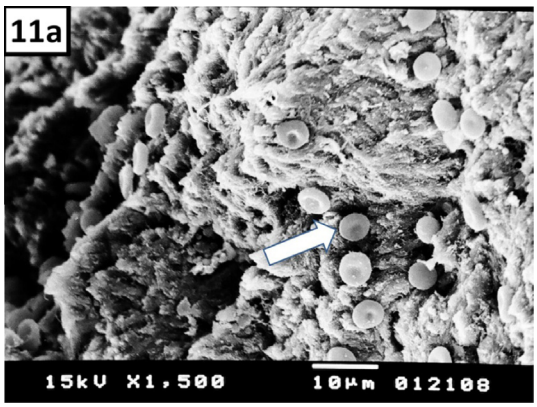


Figure 11a Organ of Corti of a rat injected with Amikacin (SEM). Scanning electron photomicrographs of the cochlear cavity in an OAE negative (deaf) rat, 6 months after injecting Amikacin: the cochlear cavity is infiltrated by many red blood cells (arrow). No details of hair cells neither of its stereocilia can be identified (Mic. Mag. ×1500).

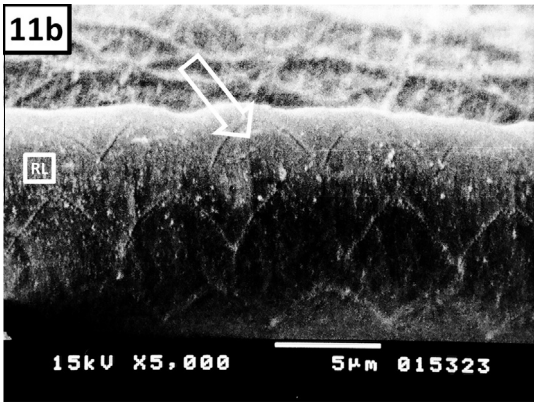


Figure 11b Organ of Corti of a rat injected with Amikacin: surface view (SEM). Surface view of the reticular lamina (RL) of the organ of Corti showing parallel rows of empty indentations (arrow) marking the lost outer hair cells (Mic. Mag. ×5000).

Parker et al. in 2004 studied the potential use of stem cells for cochlear repair.[23] Also many other stem cell studies have been done in the next few years between 2003 and 2010 but most of these studies concentrated on the transplantation of stem cells into the damaged inner ear or in vitro studies for generating hair cells from stem cells of different origins.[24–26]

The idea of mobilization of bone marrow stem cells is new and was proved to be successful in many other fields of medicine, in 2006 Zohlnhöfer et al. proved its use in acute myocardial infarction to replace the damaged cardiomyocytes.[27] Also Kloner et al. tried its use on humans in 2006 in attempts to recruit stem cells for repair of acute myocardial infarction.[28]

Mobilization of stem cells by GCSF was also utilized in acute cerebral ischemia by Schabitz et al. in 2003 and by Shyu et al. in 2006 in acute ischemic stroke.[29,30]

Also it was tried to use GCSF to mobilize bone marrow stem cells in retinal damaged photoreceptors by Oishi et al. in 2008 and in a model of Parkinson's disease by Meuer et al. in 2006 and in many other fields.[31,32]

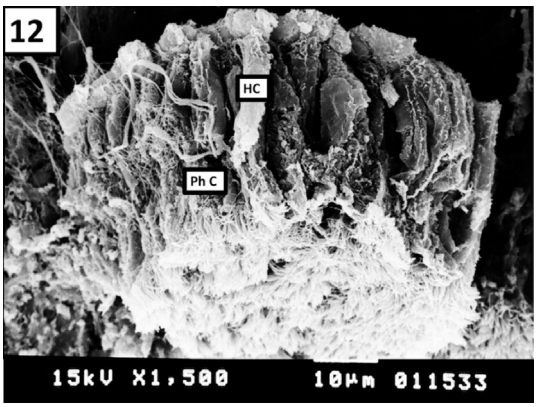


Figure 12 Organ of Corti of a rat recovering 24 weeks after GCSF (SEM). Scanning electron photomicrograph of the cochlear cavity of OAE positive rat, recovering after 24 weeks from receiving GCSF. A group of intact hair cells (HC) is depicted. The cells are supported by the underlying supporting phalangeal cells (Ph C). Some hair cells show well formed apical stereocilia (Mic. Mag. ×1500).

In this study rats were used as an animal model for the inner ear study because they are the most commonly used animal model for studying the drug effects on the inner ear due to similarities between their inner ear anatomy and human inner ear anatomy.[19–21]

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 humans which is not uncommon, so we used Electrophysiological study which is a portable Otoacoustic emission (OAE) as a screening tool for hearing function and the rats which were negative were excluded before the start of the study.

We used aminoglycosides (Amikacin sulfate) to cause cochlear damage to the rat's inner ear because it have a more cochlear side effect than vestibular unlike Streptomycin and Gentamicin which are the most commonly used drugs in the past studies but their effect is more vestibular than cochlear.

Amikacin sulfate was injected via intra-tympanic approach not via systemic approach to avoid its systemic nephrotoxic effect which may have killed our rats before the follow up period of the study, and also to allow rapid toxic effect on the inner ears. And this was carried out under microscopic magnification.[23,24]

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.

Some rats were injected with Amikacin bilaterally and others were injected unilaterally, in the rats with unilateral injection the contralateral ears were used as a control to have the same conditions and reduce the inter-animal variabilities.

We used a 5 day therapeutic regimen of subcutaneous injection of GCSF and the dose according to previous publications in the literature in humans is 5–10 µg/kg/day but in rats it is 100–200 µg/kg/day.[16]

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 months.

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).

The positive control group (Rats which were bilaterally injected with Amikacin but did not treated with GCSF) remains negative OAEs after the same follow up period of 6 months and this indicates that there was no spontaneous improvement in hearing function over this period of time.

Histological comparison was done between the inner ear of normal rats and the inner ear of Amikacin injected rats and showed that the damage was more cochlear in the form of destruction of Organ of Corti and disappearance of the inner and outer supporting cells and hair cells.

After treating Amikacin injected rats with GCSF, histological examination was done on variable durations and showed improvement in the arrangement of hair cells till we reached complete restoration of Organ of Corti after 6 months and this was consistent 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 consistent with the clinical results.

7. Conclusions and future recommendations

The use of Stem cells in hearing loss correction is a widely growing field in the last few years.

- Stem cell 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.
- 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 humans 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 must utilize more animal models and over an extended period of time.
- We also recommend the use of ABR to assess the hearing function quantitatively better than the OAEs alone.
- A measure of stem cell mobilization is also recommended to be used as blood analysis for stem cells.
- Detection of stem cells in the inner ear by immunohistochemistry is recommended to be used.

Disclosures

There are no disclosures to declare.

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