

Original Article	Effect of Amikacin on the Postnatal Development of the Cochlea in Albino Rats <i>Mohamed A. Gabr, Dorreia A. Zagloul, Faten Y. Mahmoud and Merry B. Kostandy</i> <i>Anatomy Department, Faculty of Medicine, Assiut University</i>
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ABSTRACT

Background: The cochlea is the anterior part of the labyrinth and its development is sensitive to large variety of noxious influences.

Aim of the Work: This work was conducted to study the effect of amikacin on the postnatal development of cochlea in albino rats.

Material and Methods: A total number of 30 albino rats were divided into two groups, a control group and an experimental group. The ages studied in this research were newborn, 7 days, 15 days, 1 month and 3 months postnatally in both control and experimental groups. The pregnant mothers of the experimental group received 250 mg/kg body weight of amikacin intraperitoneally daily from day 10 of gestation until the end of pregnancy. The same dose was given to the offsprings from day 1 until day 16 postnatally. The specimens were processed for histological study and stained with H&E. Ultrastructural study of the inner and outer hair cells and the large spiral ganglion cells was done through preparation of semithin and ultrathin sections. Morphometric measurements were performed to study the number of spiral ganglion cells in all the studied groups and were statistically analyzed.

Results: This study revealed that the cochlea was still immature at birth and reached the adult appearance at the age of 15 days postnatally. The cochlear structures including the organ of Corti were found to be mature from the base to the apex of the cochlea.

We observed that amikacin has no apparent effect on development of the cochlea in the newborn rats. In the 7-day-old rats, some degenerative changes appeared in the outer and inner hair cells. This effect became manifested in 15-day-old animals. Ultrastructural study of the outer hair cells showed peripheral condensation of nuclear chromatin with presence of many cytoplasmic vacuoles. At 1-month-age, there was disorganization of the organ of Corti, where atypical cells found in the region of the outer hair cells while the inner hair cells showed signs of degeneration. At the age of three months, there was complete destruction of the organ of Corti along the whole cochlea.

Morphometric study for the number of the spiral ganglion cells showed highly significant decrease in the amikacin treated animals at the age of 15- day-old rats. This decrease became very highly significant at the age of one month and three months old amikacin treated rats

Conclusions: Amikacin has a damaging effect on the organ of Corti and so considered as one of the causes of sensory deafness. The effect of amikacin was manifested during the second postnatal week is corresponding to the period of maturity of the cochlea.

Corresponding Author: Dr. Dorreia Zagloul, Anatomy Department, Faculty of Medicine, Assiut University, Assiut, Egypt, Mobile: 0105750430, e-mail: mdorria@yahoo.com.

Key Words: Amikacin, cochlea, ultrastructure, postnatal development, albino rats

INTRODUCTION

Amikacin belongs to the aminoglycoside group of antibiotics, which are bactericidal aminoglycosidic aminocyclitols (Guthrie, 2008). Today, aminoglycosides are the most commonly used antibiotics worldwide; thanks to their high efficacy and low cost (Buszman, et al. 2003). In spite

of the existence of several mechanisms of resistance, they continue to be active against most of the aerobic gram-negative bacilli (Palomino and Pachon, 2003). All aminoglycosides have a potential to induce severe and irreversible ototoxicity in the cochlea and vestibular organ. Among

them, streptomycin and gentamicin are primarily vestibulotoxic, whereas amikacin, neomycin, dihydrostreptomycin, and kanamycin are primarily cochleotoxic (Selimoglu, 2007). During treatment, high concentrations of aminoglycosides are found in the endolymph and perilymph of the inner ear where these antibiotics appear to generate free radicals, with subsequent permanent damage to the sensory cells and neurons, resulting in permanent bilateral severe high-frequency sensorineural hearing loss and temporary vestibular hypofunction (Selimoglu, 2007; Guthrie, 2008). Recent studies focused attention on age-dependent effects of ototoxic aminoglycosides and the possible mechanisms to attenuate their ototoxicity.

The aim of this work was to study the effect of amikacin on the cochlea of the albino rat during the period of postnatal development.

MATERIAL AND METHODS

In this work, a total number of 30 adult albino rats were used. They were divided into two groups, a control group and an experimental one. Separation between male and female rats was done for 20 days to be sure that they were not pregnant. Then, mating was allowed between male and female rats. The commence of pregnancy was determined by the presence of vaginal plug early in the morning and sometimes by doing vaginal smear. Pregnant females were isolated, each in a separate cage to be sure of adequate nourishment.

In the experimental group, amikacin sulphate (Amikin) was administered to the pregnant rat daily from the 10th day of gestation till birth. Then, amikacin was administered to the offsprings daily until 16 days postnatally. The dosage was 250mg/kg body weight injected intraperitoneally (Lenoir, et al. 1983). The ages studied in this work were newborn, 7 days, 15 days, 1 month and 3 months postnatally in both the experimental and control groups. The control group included the offsprings of non treated mothers.

Animals were sacrificed and their heads were dissected. Specimens of the temporal bone were embedded in paraffin and cut serially in the sagittal plane (8-10 μ in thickness). Sections were stained with haematoxylin and eosin according to the method of Carleton et al. (1980). At the ages of 15 days and 1 month postnatally, ultra-

structural study of inner and outer hair cells were done through the preparation of semithin and ultrathin sections. Ultrathin sections (450-500 A) were stained with uranyl acetate and lead citrate and examined by Jeol-JEM-100CXII electron microscopy.

Estimation of the number of spiral ganglion cells per an area of (12250 micron)² was done in both control and treated animals in all the studied groups. The numbers were estimated from the paraffin sections using computerized image analysis system Leica Q 500M.C. Comparison between the control and treated animals at different age groups was done by student's t-test.

RESULTS

Newborn rat: The cochlea was formed of two and half turns. In the basal and middle turns, the organ of Corti was developed in the form of one row of inner and three rows of outer hair cells resting on one layer of supporting cells. Inner and outer pillar cells were thin short cells with basal nuclei. They bounded a space (the tunnel of Corti). The tectorial membrane appeared as a strip of eosinophilic material extended from the limbus spiralis towards the surface of the hair cells. The limbus spiralis was formed of a layer of columnar epithelium that was overlying a region of extracellular matrix. The internal spiral sulcus (sulcus spiralis internus) was still not opened (Fig. 1). In the apical turn, the organ of Corti was still not differentiated (Fig. 3). The cells of the spiral ganglion were apparent with nerve fibers originating from them. In the amikacin treated rats, no apparent changes in organ of Corti could be identified (Figs. 2, 4).

Seven -day -old rats: In the basal and middle turns, the organ of Corti became well developed (Fig. 5). The outer hair cells were elongated than those of the previous age. Nuel's space appeared between the outer hair cells. The inner hair cells were flask in shape. Pillar cells became taller and thicker than those of the previous age and moved to the opposite sides of the tunnel of Corti. In the apical turn, the organ of Corti became differentiated and its structure was nearly similar to that of the other turns (Fig. 7). The limbus spiralis and the sulcus spiralis internus were less developed than those of the basal turn.

In the amikacin treated rats, there was widening of the Nuel's space between the outer hair cells. The cytoplasm of the inner and outer hair cells of the three cochlear turns appeared less densely stained as compared with the control (Figs. 6, 8).

Fifteen-day-old rats: The organ of Corti became mature in all turns of the cochlea. Ultrathin sections showed that the outer hair cell is cylindrical in shape with basally located euchromatic nucleus and prominent nucleolus. The cytoplasm was rich in mitochondria and free ribosomes. In its apical part, the cuticular plate was observed in which the stereocilia were implanted (Fig. 9). The inner hair cell was flask shaped (Fig. 11) with basally located nucleus. The cytoplasm showed the presence of mitochondria, free ribosomes and some vesicles. The apical surface showed the presence of stereocilia. The spiral ganglion was formed of large rounded and small rounded or fusiform cells (Fig. 23) with many nerve fibers extending from them toward the organ of Corti.

In the amikacin treated rat, the ultrathin sections showed condensation of the chromatin at the periphery of the nucleus of the outer hair cell with many cavities and dense bodies within the cytoplasm. There was loss of stereocilia at the surface of the cell (Fig. 10). The cytoplasm of the inner hair cell had many vacuoles and dense bodies. The neck of the cell appeared to be constricted at its apical pole (Fig. 12).

The spiral ganglion in the treated rat showed decreased cell density in comparison with the control (Fig. 24).

One-month-old rats: The cochlea reached the full maturity. The organ of Corti was well developed (Fig. 13). The tunnel of Corti was well apparent between the inner and outer pillar cells. The tectorial membrane was well developed.

Ultrathin sections showed normal appearance of the outer and inner hair cells as seen in the previous age. The large spiral ganglion cells showed large nucleus with evenly distributed chromatin and prominent nucleolus. The cytoplasm was rich with organelles including parallel cisterns of rough endoplasmic reticulum, free ribosomes, Golgi apparatus and mitochondria (Fig. 27).

In the treated rat, the organ of Corti in the basal turn was disorganized (Fig. 14). The outer hair cells were lost and their region showed the presence of small cells with rounded nuclei. The inner hair cells could be identified. The tunnel of Corti appeared to be collapsed as compared with the control. In the middle and apical turns, the organ of Corti showed similar changes with less collapsed tunnel of Corti (Fig. 15).

Ultrathin sections showed small oval cells (atypical cells) in the region of the outer hair cells that were lost. These small cells had dark cytoplasm that showed the presence of ribosomes, rough endoplasmic reticulum and mitochondria. Gap junctions were present on their lateral surface (Fig. 16). Their apical surface showed the presence of microvilli with rounded tips (Fig. 17). The inner hair cells showed many large vacuoles within their cytoplasm (Fig. 18).

Ultrathin sections showed some defects in the nuclear membrane of the large spiral ganglion cells. Their cytoplasm had many lysosomes with clumped rough endoplasmic reticulum cisterns and apparent decrease in ribosomes as compared with the control (Fig. 28).

Three-month-old rats: The structure of the cochlea was similar to the previous age (Fig. 19). The spiral ganglion showed an increase in the density of nerve cells and fibers (Fig. 25).

In the treated rat, the organ of Corti was completely destroyed in the basal turn (Fig. 20), while it was markedly disorganized in the middle (Fig. 21) and apical turns (Fig. 22). The spiral ganglion showed apparent decrease in the cells and nerve fibers (Fig. 26).

Morphometric results: There was a decrease in the mean number of the spiral ganglion cells in all treated groups as compared with the control (Table 1; Fig. 29). This decrease was not statistically significant at the age of newborn and 7 days old animals while it was highly significant ($P < 0.005$) in the 15 days old animals. In one month and three months old treated animals, the mean number of the spiral ganglion cells showed very high significant decrease ($P < 0.001$) as compared with those of the control.

Table 1: Number of spiral ganglion cells per an area of 12250 μm^2 .

Readings	Control group		Treated group				t	
	N	Mean	SE	N	Mean	SE		Difference
Newborn	6	85.1667	4.7005	6	84.6667	2.7325	0.5	0.0848
7 days	6	84.1667	2.6885	6	79.8333	3.5559	4.3333	0.9659 **
15 days	6	83.8333	1.9047	6	69.1667	2.9712	14.6667	4.1558 ***
1 month	6	92.1667	6.4983	6	42.5	1.8394	49.6667	7.354 ***
3 months	6	100.6667	3.5371	6	32.8333	1.4472	67.8333	17.749 ***

N → Number. SE → standard error.
 Significance probability: $P < 0.01 \rightarrow (*)$ significant difference
 $P < 0.005 \rightarrow (**)$ high significant difference.
 $P < 0.001 \rightarrow (***)$ very high significant difference

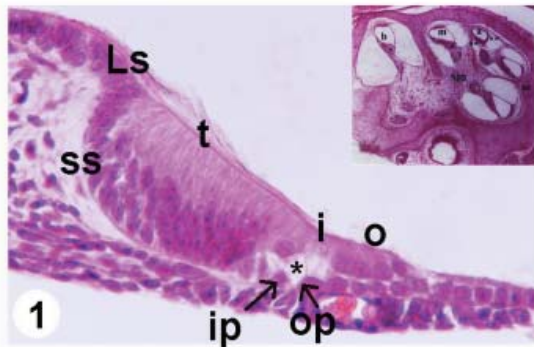


Fig. 1: A photomicrograph of the basal turn of the cochlea in newborn control rat showing one inner (i) and three outer (o) hair cells, inner pillar (ip) and outer pillar (op) cells separated by the tunnel of Corti (*) with the internal spiral sulcus (ss) still not opened. Note the limbus spiralis (Ls) and the tectorial membrane (t).

Inset: Turns of the cochlea basal (b), middle (m) and apical (a) turns. Hx. & E.; X400

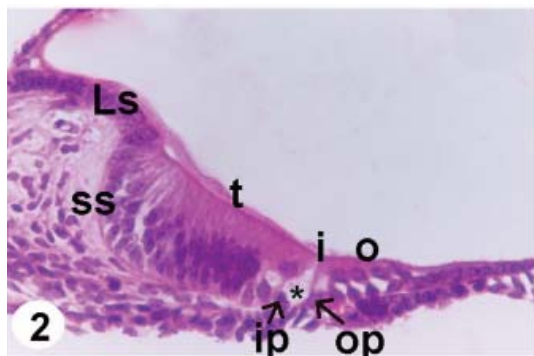


Fig. 2: A photomicrograph of the basal turn of the cochlea in newborn treated rat showing no apparent effect of amikacin. Notice inner hair cell (i), inner pillar cells (ip), limbus spiralis (Ls), outer hair cell (o), outer pillar cells (op), internal spiral sulcus (ss) and tectorial membrane (t). Hx. & E.; X400

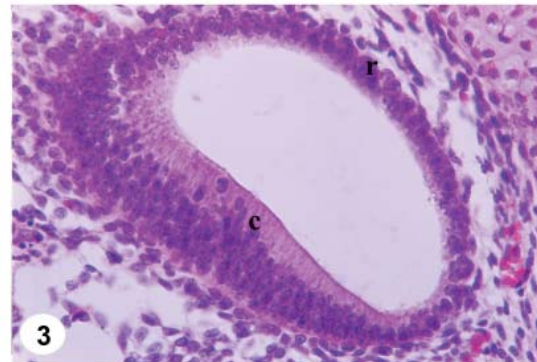


Fig. 3: A photomicrograph of the apical turn of the cochlea in newborn control rat showing rostral wall (r) and caudal wall (c) with no differentiation of organ of Corti. Hx. & E.; X400

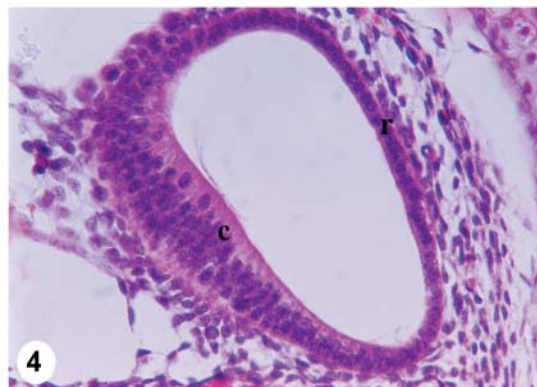


Fig. 4: A photomicrograph of the apical turn of the cochlea in the newborn treated rats showing no apparent effect of amikacin. Note the caudal wall (c) and rostral wall (r). Hx. & E.; X400

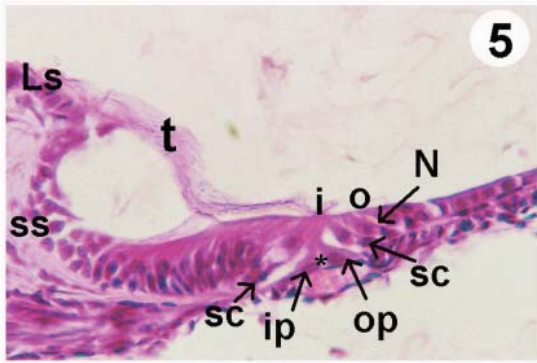


Fig. 5: A photomicrograph of the basal turn of the cochlea in 7-day-old control rats showing more developed organ of Corti, flask shaped inner hair cells (i), elongated outer hair cells(o) with appearance of Nuel's space (N) and opened internal spiral sulcus (ss). Note inner pillar cells (ip), limbus spiralis (Ls), outer pillar cells (op), tectorial membrane (t), the tunnel of Corti (*) and the supporting cells (sc).
Hx. & E.;X400



Fig. 6: A photomicrograph of the basal turn of the cochlea in 7-day-old treated rat showing lightly stained cytoplasm of the inner (i) and outer hair cells(o).Note inner pillar cells (ip), outer pillar cells (op), tectorial membrane (t), tunnel of Corti (*) and the supporting cells (sc).
Hx. & E.;X400



Fig. 7: A photomicrograph of the apical turn of the cochlea in 7-day-old control rat showing differentiation of organ of Corti and less developed limbus spiralis (Ls) and the sulcus spiralis internus (ss) than those of the basal turn. Note inner hair cell (i), inner pillar cells (ip), outer hair cell (o), outer pillar cells (op), tectorial membrane (t), tunnel of Corti (*) and the supporting cells (sc).
Hx. & E.;X400



Fig. 8: A photomicrograph of the apical turn of the cochlea in 7-day-old treated rat showing lightly stained cytoplasm of the inner(i) and outer hair cells (o) with relative widening of the Nuel's spaces (N). Note inner pillar cells (ip), outer pillar cells (op), tectorial membrane (t), tunnel of Corti (*) and the supporting cells (sc).
Hx. & E.;X400

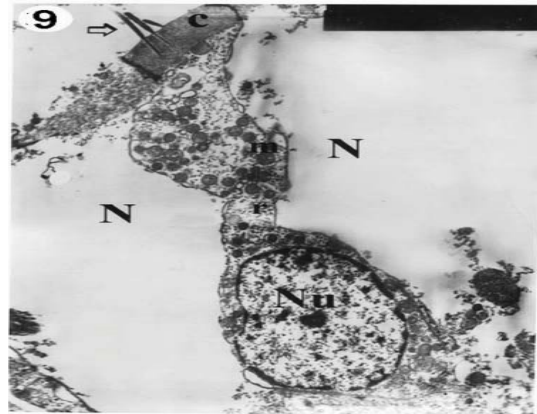


Fig. 9: An electron photomicrograph of the outer hair cell of the cochlea in 15-day-old control rat showing basally located euchromatic nucleus (Nu) with well developed mitochondria (m) and free ribosomes(r).The apical part of the cell showed the presence of the cuticular plate (c) in which stereocilia (arrow) were implanted . Note Nuel space (N).
X5000

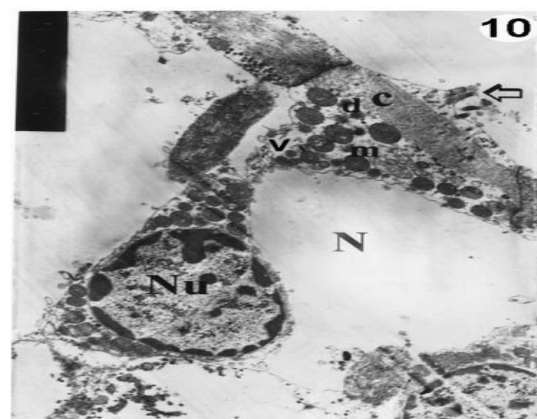


Fig. 10: An electron photomicrograph of the outer hair cell of the cochlea in 15-day-old treated rat showing condensation of chromatin at the periphery of the nucleus (Nu), with cavitations (v) and dense bodies (d) in the cytoplasm and apparent loss of stereocilia (arrow). Note cuticular plate (c).
X5000

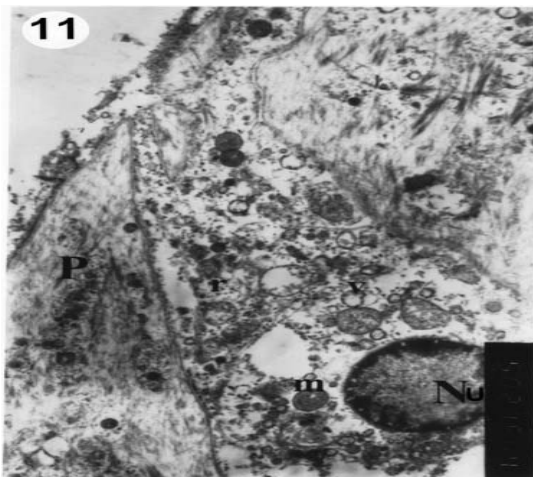


Fig. 11: An electron photomicrograph of inner hair cell of the cochlea in 15-day-old control rat showing basally located nucleus (Nu) with many mitochondria (m) and free ribosomes (r) and some vesicles (v) in cytoplasm. Note pillar cell (P). X5000

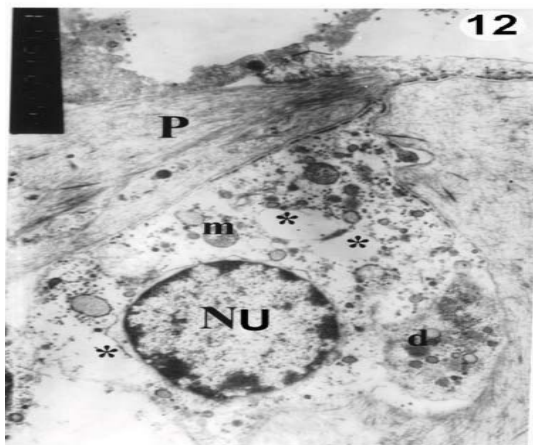


Fig. 12: An electron photomicrograph of inner hair cell of the cochlea in 15-day-old treated rat showing vacuoles(*) and dense bodies (d) within the cytoplasm. The cell appeared to be constricted at its apical pole. Note mitochondria (m), nucleus (Nu) and pillar cell (P). X5000

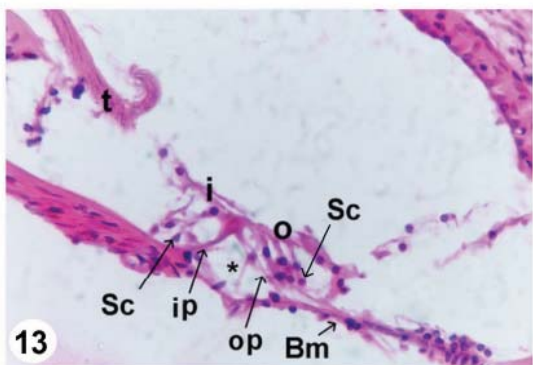


Fig. 13: A photomicrograph of the basal turn of the cochlea in one-month-old control rat showing the organ of Corti, with full maturity, resting on the basilar membrane (Bm). Note inner hair cells (i), inner pillar cells (ip), outer hair cells (o), outer pillar cells (op), supporting cells (sc), tectorial membrane (t) and tunnel of Corti (*). Hx & E.; X400

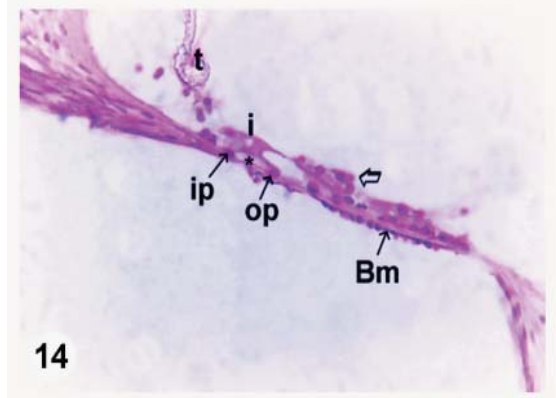


Fig. 14: A photomicrograph of the basal turn of the cochlea in one-month-old treated rat showing markedly disorganized organ of Corti with the appearance of small cells with rounded nuclei (arrow) in the place of the outer hair cells, lightly stained inner hair cells (i) and collapsed tunnel of Corti (*). No apparent change could be observed in the basilar membrane (Bm). Hx & E.; X400



Fig. 15: A photomicrograph of the apical turn in the cochlea of one-month-old treated rat showing similar changes to those of the basal turn with less collapsed tunnel of Corti (*). Note atypical cells (arrow), basilar membrane (Bm), inner hair cells (i), inner pillar cells (ip); and outer pillar cells (op). Hx & E.; X400

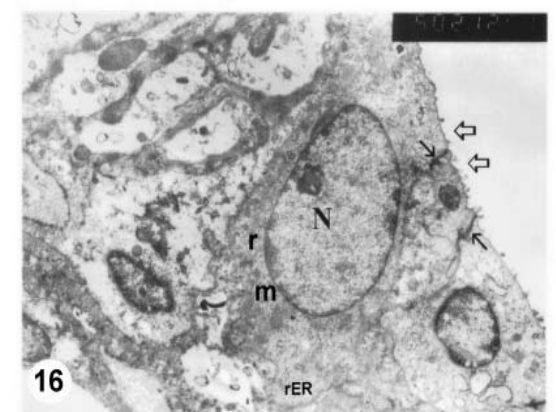


Fig. 16: An electron photomicrograph of the atypical cells present in the cochlea of one-month-old treated rat showing dark cytoplasm with rough endoplasmic reticulum (rER), ribosomes (r) and mitochondria (m). Gap junctions (thin arrows) were present on their lateral surfaces. The apical surface showed the presence of microvilli with rounded tips (thick arrows). X 5000

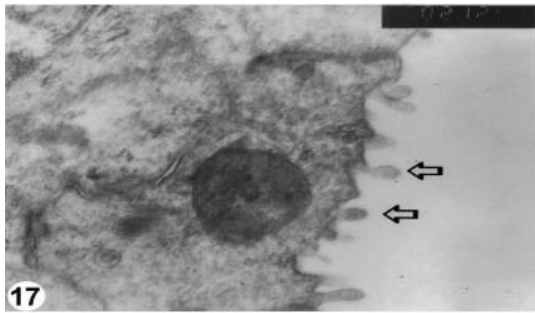


Fig. 17: Higher magnification of part of the previous photomicrograph showing the microvilli (arrows) of the atypical cell. X20000

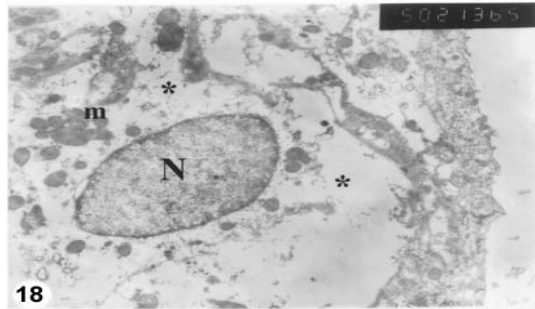


Fig. 18: An electron photomicrograph of the inner hair cell of the cochlea in one-month-old treated rat showing large vacuoles (*) and damaged mitochondria (m). X 5000

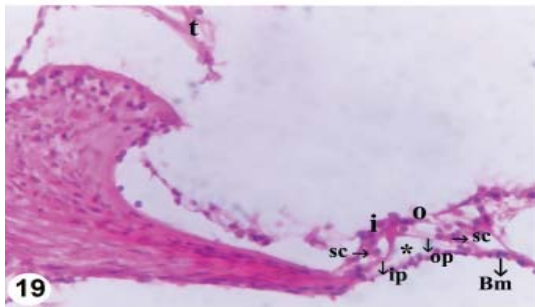


Fig. 19: A photomicrograph of the basal turn of the cochlea in 3-month-old control rat showing the organ of Corti resting on the basilar membrane (Bm). Note inner hair cells (i), inner pillar cells (ip), outer hair cells (o), outer pillar cells (op), supporting cells (sc), tectorial membrane (t) and tunnel of Corti (*). Hx. & E.; X 400

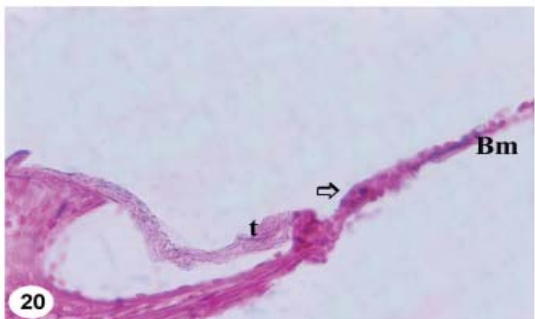


Fig. 20: A photomicrograph of the basal turn of the cochlea in 3-month-old treated rat showing complete destruction of the organ of Corti (arrow). Note basilar membrane (Bm) and tectorial membrane (t). Hx. & E.; X 400

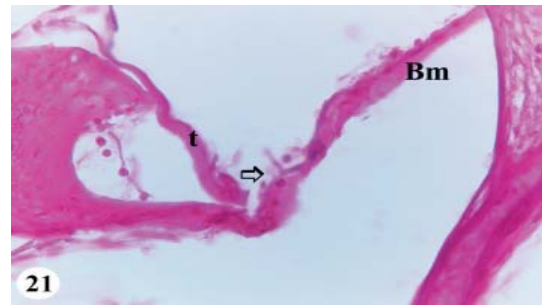


Fig. 21: A photomicrograph of the middle turn of the cochlea in 3-month-old treated rat showing marked disorganization of the organ of Corti (arrow). Note basilar membrane (Bm) and tectorial membrane (t). Hx. & E.; X 400

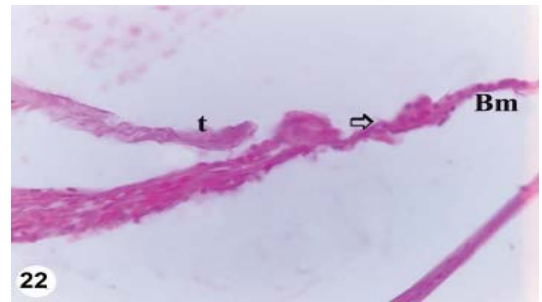


Fig. 22: A photomicrograph of the apical turn of the cochlea in 3-month-old treated rat showing marked disorganization of the organ of Corti (arrow). Note basilar membrane (Bm) and tectorial membrane (t). Hx. & E.; X 400

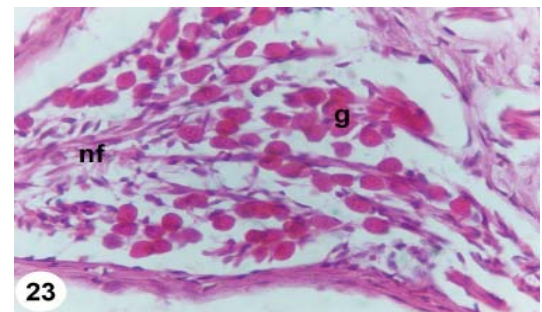


Fig. 23: A photomicrograph of the spiral ganglion in the Rosenthal's canal of the cochlea in 15-day-old control rat showing large and small ganglion cells (g). Note nerve fibers (nf). Hx. & E.; X 400

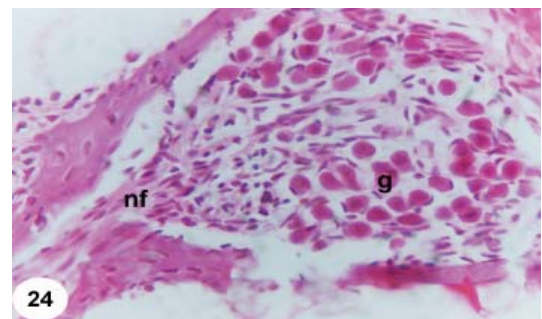


Fig. 24: A photomicrograph of the spiral ganglion in the Rosenthal's canal of the cochlea in 15-day-old treated rat showing some decrease in the density of ganglion cells (g), not nerve fibers (nf). Hx. & E.; X 400

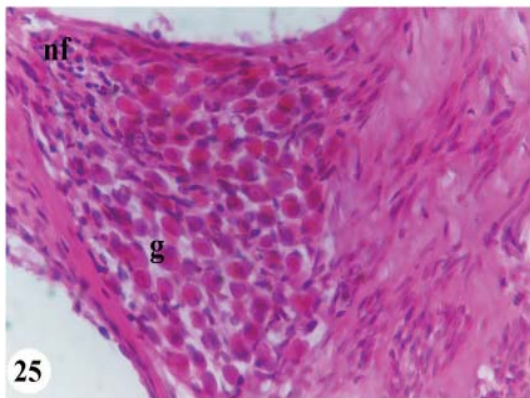


Fig. 25: A photomicrograph of the spiral ganglion of 3-month-old control rat showing an increase in the ganglion cells(g) and nerve fibers(nf) than those of the previous age. Hx. & E.; X 400

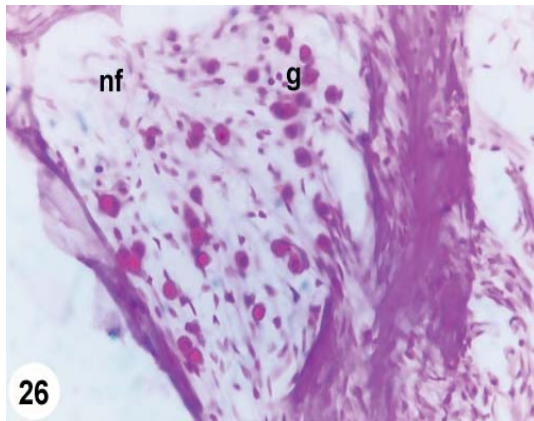


Fig. 26: A photomicrograph of the spiral ganglion of 3-month-old treated rat showing marked decrease in the density of ganglion cells (g) and nerve fibers (nf) in comparison with the control. Hx. & E.; X 400

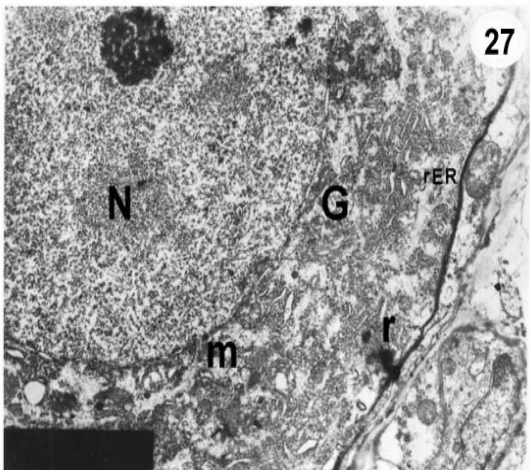


Fig. 27: An electron photomicrograph of a large spiral ganglion cell of one-month-old control rat showing centrally located nucleus (N) with evenly distributed chromatin and prominent nucleolus. The cytoplasm showed the presence of rough endoplasmic reticulum (rER), ribosomes (r), Golgi apparatus (G) and mitochondria (m). X5000

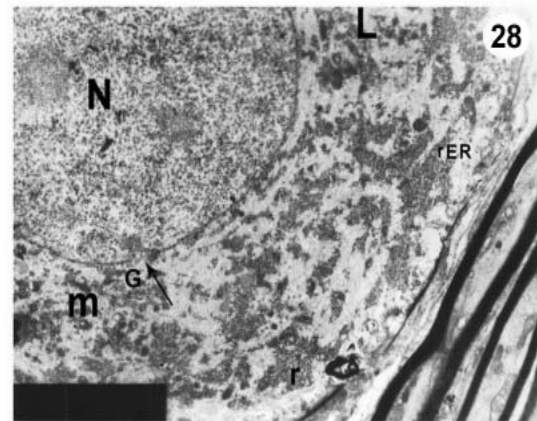


Fig. 28: An electron photomicrograph of a large spiral ganglion cell of one-month-old treated rat showing disruption of the nuclear membrane (arrow) and clumped rough endoplasmic reticulum cisterns (rER) with apparent decrease in the free ribosomes (r) as compared with those of the control. Note Golgi apparatus (G), lysosomes (L), mitochondria (m) and nucleus (N). X5000

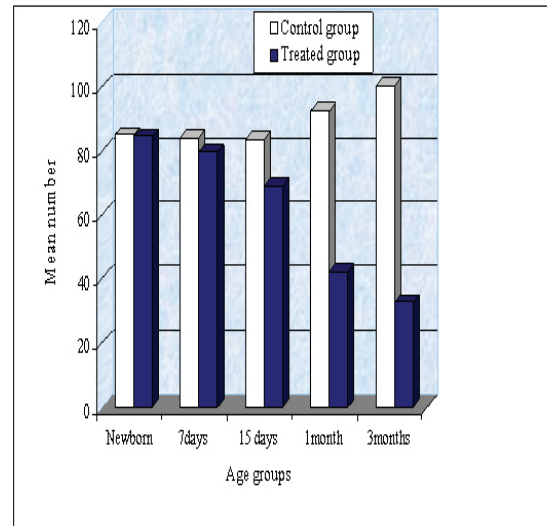


Fig. 29: Mean number of ganglion cells per an area of 12250 μm^2 among different age groups.

DISCUSSION

The present study revealed developmental changes in the cochlea of albino rat after birth. At birth, the cochlea was formed of two and half turns but still immature. In the basal and middle turns, the organ of Corti was developed in the form of one row of inner and three rows of outer hair cells resting on one layer of supporting cells. However in the apical turn, it was still not differentiated. Differentiation of the hair cells was apparent in the apical turn on the 7th day postnatal. This follows the general gradient of maturation from the base to the apex (Pujol, et al. 1991). These results are

in harmony with those of *Lenoir and Puel (1987)* who described very immature organ of Corti in the 2-day-old rat. These findings are also supported by *Uziel et al. (1981)* and *Pujol (1986)* who studied the functional maturation of rat cochlea using electrophysiological recordings of microphonic potential and found that the rat cochlea is fully immature at birth.

In the following postnatal age groups, the organ of Corti showed marked changes. The outer hair cells became cylindrical in shape and the inner hair cells enlarged in size. The tunnel of Corti, the space of Nuel, the sulcus spiralis internus and the limbus spiralis were developed in the basal turn by the 7th postnatal day. The organ of Corti completed its development in the middle and apical turns in the 2nd postnatal week. These findings are in accordance with *Lenoir et al. (1980)* who observed maturation of the organ of Corti and its surrounding structures in the three turns of the rat cochlea at postnatal day 16. However, *Roth and Bruns (1992)* observed the adult appearance of the cochlear structures in the basal turn by the 16th day after birth while complete development of the apical region was delayed 4-5 days after that. Moreover, *Mu et al. (1997)* reported that the adult size of inner and outer hair cells was obtained by 7-14 days postnatally in rat. In accordance with that, *Pujol and Hilding (1973)* stated that the second postnatal week is the period of onset of cochlear function in rats.

In agreement with *Standring et al. (2004)*, the present study showed the presence of stereocilia on the surface of the hair cells. *Kelly and Chen (2007)* reported that this apical specialization of the hair cells was essential for mechano-transduction and for frequency selection.

According to the effect of amikacin on the cochlea in the present study, no changes could be detected in the organ of Corti by light microscopic examination up to 15-day-old rats. However, electron microscopic examination demonstrated signs of degeneration of the outer and inner hair cells in 15-day-old treated rats. These were in the form of peripheral condensation of nuclear chromatin, vesicles and dense bodies in the cytoplasm and lost stereocilia. These results are in agreement with *Onejeme and Khan (1984)* who found that the cochleae of rat fetuses exposed in utero to aminoglycosides and those of neonates exposed from day 1 to day 8 postnatally were unaffected.

Moreover, *Pujol (1986)* found that the susceptibility to aminoglycoside ototoxicity commenced around the period of functional maturation. This period lies between the onset of function and the acquisition of adult properties. It lies between the 8th and 16th postnatal days in rats, during which the last stages of inner and outer hair cells ciliogenesis and the maturation of the under surface of the tectorial membrane occur (*Lenoir; et al. 1987*). In addition, *Vago et al. (1998)* observed that the 14th postnatal day in rat corresponded to the period of supranormal sensitivity to aminoglycoside antibiotics. In human fetus, the auditory function commences between 19 and 27 weeks of gestation (*Hepper and Shahidullah, 1994*).

At one-month-old treated rats, the present study revealed disorganization of the organ of Corti that was more severe in the basal turn than that of the middle and apical turns. This coincides with *Chambers (2006)* who reported that the damage proceeded from the base to the apex. In the present work, the outer hair cells were lost and replaced by atypical cells while the inner hair cells were still present. This indicates that the inner hair cells are more resistant to damaging effect of the aminoglycosides than the outer hair cells (*Lenoir and Puel, 1987*). Besides, *Raphael (2002)* suggested that cochlear supporting cells actively participated in the process of hair cell elimination and scar formation.

In the present work, Ultrathin sections of the atypical cells showed a dark nucleus and dense cytoplasm with microvilli instead of stereocilia on the apical surfaces in relation to the endolymphatic compartment. Moreover, gap junctions were observed between the atypical cells and the neighboring Dieter's cells. These results are in harmony with those of *Lenoir and Vago (1996)* who found atypical cells among the Deiter's cells expansions in 35-day-old treated rat given amikacin during the period of cochlear supranormal sensitivity. *Daudet et al. (1998)* explained the presence of gap junctions to a specific feature of cochlear non-sensory cells. On the other hand, *Gulley and Reese (1976)* reported that the gap junctions were never observed at the lateral membranes of the cochlear hair cells.

In the present study at the age of 3-month-old treated rat, the organ of Corti was completely destroyed and the basilar membrane was covered by undifferentiated cells. No regeneration of the hair

cells was observed. These results coincide with results reported by *Lenoir et al. (1999)* who found that the organ of Corti was reduced to an undifferentiated epithelium throughout the three cochlear turns of 90-day-old rat. This is also supported by *Vago et al. (1998)* who reported that lost hair cells were not replaced by new ones in the mammalian cochlea. However, in the auditory system of avian and other lower vertebrates lost hair cells were replaced by new hair cells throughout regeneration (*Roberson, et al. 1992*).

Daudet et al. (1998) explained disappearance of the atypical cells by considering that antibiotics remained in the cochlear tissues at a high concentration for a long time and it is possible that the atypical cells themselves suffered the deleterious effect of the antibiotic.

Sorimachi (2000) attributed the aminoglycosides ototoxicity to their ability to produce an increase in the intracellular calcium level which in turn activates calpain (calcium-dependant cysteine protease). Its activation breaks down the cytoskeletal and membrane proteins, kinases and transcription factors conclusively leading to hair cell death. Moreover, *Dulon et al. (1991)* found that the lesions, caused by the increase in the intracellular calcium levels, occurred preferentially in the outer hair cell.

The present morphometric study showed gradual decrease of the spiral ganglion cells in the amikacin treated animals with the progress of age. This decrease was found to be significant at the age of 15 days and became very highly significant in one -and three- month-old rats. Ultrastructural study of the large spiral ganglion cells at one month showed apparent degenerative changes. These findings are in agreement with *Koitchev et al. (1982)* who observed degeneration and considerable loss of 30 to 55% of ganglion cells at one month after massive treatment with amikacin in the guinea pig. They observed continuous loss of the spiral ganglion cells which reached up to 85% after one year. They suggested that this rapid degeneration might be due to the direct influence of toxic substances on the ganglion cells. *Stankovic et al. (2004)* attributed the loss of the spiral ganglion cells to their deprivation from the neurotrophic support produced by the hair cells which when lost led to secondary degeneration of ganglion cells.

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تأثير الاميكاسين على نمو قوقعة الأذن بعد الولادة في الفئران البيضاء

محمد عبد المغنى جبر، درية عبدالله زغلول، فاتن يوسف محمود، ميرى بنيامين قسطندي

قسم التشريح - كلية الطب - جامعة أسيوط

ملخص البحث

قوقعة الأذن هي الجزء من الأذن الداخلية المسئول عن السمع ويعتبر عضو كورتي هو المستقبل الرئيسي لحاسة السمع. تهدف هذه الدراسة الى بحث تأثير الاميكاسين على نمو القوقعة في مرحلة ما بعد الولادة في الفئران البيضاء.

أستخدم في هذا البحث عدد ثلاثون من الفئران البيضاء حيث قسموا الى مجموعتين (مجموعة ضابطة ومجموعة تجريبية). أعطيت الامهات الحوامل في المجموعة التجريبية جرعة من الاميكاسين تعادل 250 مجم/كجم من وزن الجسم يوميا عن طريق الحقن في التجويف البريتوني. تم اعطاء الجرعة من اليوم العاشر من الحمل وحتى الولادة ثم اعطاء الفئران الوليدة نفس الجرعة من اول يوم للولادة وحتى اليوم 16 بعد الولادة. وقد اجريت هذه الدراسة على خمس مجموعات عمرية وهي الفئران حديثي الولادة، سبعة ايام، خمسة عشر يوم، شهر، ثلاثة شهور بعد الولادة. تمت التضحية بالحيوانات في نهاية التجربة وتجهيز عينات في كل مجموعة لدراستها هستولوجيا بعد صبغها بالهيماتوكسيلين والايوسين. كذلك تمت دراسة التركيب الدقيق للخلايا المشعرة الداخلية والخلايا المشعرة الخارجية والخلايا الكبيرة للعقدة الحلزونية عن طريق تجهيز قطاعات نصف رقيقة وقطاعات فائقة الرقة. وقد اجريت ايضا دراسة مورفومترية اشتملت على قياس عدد الخلايا في العقدة العصبية السمعية في مساحة (تعادل 2250 ميكرون مربع) داخل القوقعة في جميع الاعمار التي تمت دراستها و اجراء التحليل الاحصائي لها.

أوضحت نتائج هذا البحث ان القوقعة لا تزال غير كاملة النمو في اليوم الاول بعد الولادة ووجد أنها تصل الى مرحلة النضوج التام عند عمر خمسة عشرة يوما. وان نمو مكونات القوقعة يبدأ من اللفة القاعدية ثم يمتد للقمّة.

وقد تبين أن الاميكاسين ليس له تأثير على نمو القوقعة في الفئران حديثي الولادة ولكن ظهر هذا التأثير واضحا عند عمر خمسة عشر يوما بعد الولادة حيث أظهر الفحص الدقيق للخلايا المشعرة الخارجية وجود علامات انحلال بالخلايا واستمر هذا التأثير حتى عمر شهر حيث اختفت الخلايا المشعرة الخارجية وظهرت خلايا غير نمطية بدلا منها، اما الخلايا المشعرة الداخلية فقد ظهرت بها علامات انحلالية عند عمر خمسة عشرة يوما مع استمرار وجودها حتى عمر شهر. اما عند عمر ثلاثة شهور فقد ظهر ان هناك تدميراً كاملاً لعضو كورتي في القوقعة بكاملها.

وكذلك اوضحت الدراسة المورفومترية وجود نقصا ذا دلالة احصائية في عدد خلايا العقدة الحلزونية للقوقعة في الحيوانات المعالجة بالاميكاسين عند عمر خمسة عشر يوما و شهر وثلاثة شهور بعد الولادة مقارنة بنفس الاعمار في المجموعات الضابطة.

يتضح من هذه الدراسة ان الاميكاسين له تأثير مدمر على عضو كورتي لذلك يمكن اعتباره احد اسباب الصمم الحسى. وقد ظهر واضحا هذا التأثير في فترة وصول القوقعة للنضوج.