

# Morphological Changes Induced by Three Aminoglycosides on the Cochlear Stria Vascularis.

MSc. Daymara Mercerón-Martínez,<sup>1</sup> MSc. Sandra Rodríguez-Salgueiro,<sup>2</sup> Dra. Rosa María Coro-Antich,<sup>3</sup>

MSc. Pavel Prado-Gutiérrez,<sup>1</sup> Dra. Valia Rodríguez-Rodríguez,<sup>1</sup> Dra. Lídice Galan García,<sup>1</sup>

Ing. Yahima Harvey-Pedroso,<sup>2</sup> Ing. Tania Valdés-Prieto<sup>2</sup>

## Resumen

Los antibióticos aminoglucósidos son antimicrobianos eficaces de uso común en la práctica clínica. Sus efectos sobre las células del órgano de Corti y las neuronas del ganglio espiral se han estudiado ampliamente. Sin embargo, sólo hay pocos informes sobre los cambios morfológicos inducidos por aminoglucósidos en la estría vascular. El objetivo de este estudio fue describir los cambios morfológicos y morfométricos que se producen en la estría vascular de ratas después de una pérdida auditiva sensorineural profunda inducida por tres aminoglucósidos (kanamicina, gentamicina y amikacina), seguidos por furosemida. Para analizar el área de la estría vascular y el número de capilares sanguíneos por estría vascular, se procesaron cócleas de catorce ratas a las ocho semanas de sordera y de cuatro animales controles. Se examinaron secciones semifinas cocleares de las vueltas apical, medial superior, medial inferior y basal, mediante un microscopio óptico. El grado de daño coclear dependió del aminoglucósido. Las áreas de la estría vascular en los grupos que recibieron kanamicina y gentamicina fueron menores que en los controles. El número de capilares sanguíneos promedio por estría vascular se redujo en los tres grupos de animales sordos, en comparación con los animales controles. Para ambas variables, el daño más grave se observó en los animales ensordecidos con gentamicina, seguido de kanamicina y amikacina.

**Palabras clave:** aminoglucósidos, cóclea, estría vascular, capilares sanguíneos; ototoxicidad

## Abstract

Aminoglycosides are efficient antimicrobials commonly used in clinical practice. Their effects on the cells of the organ of Corti and the spiral ganglion neurons have been extensively studied. However, there are only a few reports concerning aminoglycoside-induced morphological changes on the stria vascularis. The purpose of this study was to describe morphological and morphometrical changes on the rat stria vascularis after a profound sensorineural hearing loss induced by three aminoglycosides (kanamycin, gentamicin and amikacin) followed by furosemide. To analyze the stria vascularis area and the number of blood capillaries per stria vascularis, cochleae from fourteen rats sampled at eight weeks after deafness and from four control animals were processed. Serial semi thin cochlear sections from the apical, upper middle, lower middle and basal turns were examined under a light microscope. The cochlear damage degree depended on the aminoglycoside. Mean stria vascularis areas for both kanamycin and gentamicin groups were lower than controls. The mean number of blood capillaries per stria vascularis was reduced for the three aminoglycoside-deafened groups as compared to control animals. For both variables, the most severe damage was observed for gentamicin-deafened animals, followed by kanamycin and amikacin.

**Key words:** Aminoglycosides, cochlea, stria vascularis, blood capillaries; ototoxicity

Rev. Ecuat. Neurol. Vol. 22, N° 1-3, 2013

## Introduction

Hearing loss commonly originates as a result of irreversible damage to cochlear hair cells caused by therapeutic agents such as aminoglycoside antibiotics or cisplatin, as well as by aging, loud sounds, infections, or mechanical injury.<sup>1</sup> Despite their ototoxicity, aminoglycosides are still frequently used in clinical practice for their high bactericidal effect, as well as for their efficacy to pre-

vent infections associated with chronic diseases such as tuberculosis, cystic fibrosis and Duchenne muscular dystrophy.<sup>2</sup> In mammals, the ototoxic effect of these drugs induces a permanent sensorineural hearing loss due to the lack of regenerative capacity of cochlear hair cells.<sup>3</sup> The loss of hair cells disrupts the transduction of acoustic information into neural signals during hearing and leads to a progressive auditory degeneration that results in the loss

<sup>1</sup> Departamento de Neurociencias Experimental, Centro de Neurociencias de Cuba

<sup>2</sup> Laboratorio de Microscopía Electrónica, Dirección de Biotecnología, Centro Nacional de Investigaciones Científicas, La Habana, Cuba

<sup>3</sup> Departamento de Patología, Instituto de Neurología y Neurocirugía, La Habana, Cuba

Correspondencia

MSc. Sandra Rodríguez- Salgueiro

Laboratorio de Microscopía Electrónica, Dirección de Biotecnología, Centro Nacional de Investigaciones Científicas, Apto. Postal 6412, La Habana, Cuba.

E-mail: sandra.rodriguez@cnic.edu.cu

of spiral ganglion neurons and their peripheral processes innervating the organ of Corti (OC).<sup>4</sup> Such neuronal degeneration is particularly severe in cochlear regions where hair cells and supporting cells are missing, suggesting that they should play an important role for neuronal survival.<sup>5</sup>

The effects of several aminoglycosides on cochlear hair cells and neurons have been extensively studied.<sup>6-8</sup> Most of these studies have described a morphological base-to-apex toxicity gradient which widely varies depending on the specific aminoglycoside.<sup>9</sup> However, it is unknown if in other important cochlear structures such as the stria vascularis (SV), the toxicity differs depending on the specific aminoglycoside used.

The SV plays an important role in audition because it is responsible for the generation of the endocochlear potential, which provides the driving force for the mechanoelectrical transduction of cochlear hair cells.<sup>10,11</sup> Some studies have shown the changes that occur in the SV of mice and guinea pigs after a profound deafness due to the co-administration of kanamycin and loop diuretics (furosemide, ethacrynic acid or bumetanide). These authors described a significant degeneration of the SV following long-term ototoxicity.<sup>12,13</sup> However, little is known about the effect of aminoglycoside ototoxicity on the SV blood capillaries, which are located between the marginal and the intermediate cell layers of the SV. These capillaries provide nutritional support for the highly metabolically active cells of the SV that are responsible for the generation and maintenance of the endocochlear potential.<sup>10,14</sup>

The purpose of this study was to describe morphological and morphometrical changes on the rat stria vascularis, with emphasis on blood capillaries, after a profound sensorineural hearing loss induced by three aminoglycosides (kanamycin, gentamicin and amikacin) followed by furosemide.

### Materials and methods

Eighteen male Wistar rats weighing 250-300 g, purchased at The National Center for Laboratory Animal Production (CENPALAB) (Habana, Cuba) were used in this investigation. The animals were maintained on a 12/12 light/dark cycle, and allowed free access to food and water throughout the whole experiment. Before any experimental manipulation, all animals were tested to have normal auditory function by recording their auditory brainstem responses (ABRs). Normal hearing animals were randomly assigned to four groups of four to five rats each: three groups of deafened rats (Kanamycin and Amikacin: n=5; Gentamicin: n=4) and one group of control, non-deafened animals (n=4). All procedures were in accordance with the European Union Guidelines for Animal Experimentation as well as with the CENPALAB guidelines. They were approved by the Animal Research and Ethics Committee of the Cuban Neuroscience Center.

### Deafening Procedure

Each animal received an intravenous injection of the loop diuretic furosemide (150 mg/kg, Jiangsu Pengyao Pharmaceutical Co) followed by an aminoglycoside delivered subcutaneously. The doses of aminoglycosides were: kanamycin monosulphate 700 mg/kg (AICA); gentamicin monosulphate 420 mg/kg (AICA) and amikacin monosulphate 700 mg/kg (Quimefa). The loop diuretic facilitates the penetration of aminoglycosides into endolymph, potentiating the action of them.<sup>12,15</sup> All the animals survived after the deafening treatments.

### Auditory Brainstem Responses

Animals were anaesthetized with a single intraperitoneal injection of 75 mg/kg ketamine and 5 mg/kg diazepam. ABRs were recorded using stainless-steel needle electrodes: the positive electrode on the vertex, the negative on the neck and the ground on the abdomen. Broad band clicks of 0.1 ms were presented mono-aurally at a rate of 25 Hz using an Audix-4 stimulation-recording system (Neuronic S.A.). The signal was amplified 105 and band-pass filtered (0, 5 Hz - 2 kHz). One thousand responses were averaged for each recording and stored for subsequent analysis. Recordings were performed before and a week after the ototoxic treatment. Previous to the deafness induction, responses from both ears were obtained to a decreasing series of intensities starting from 70 dB SPL (sound pressure level) to the threshold. Threshold was defined as the smallest click amplitude required evoking a response within a latency window of 2.25-3.25 ms following the stimulus onset (wave III of the ABRs). Animals with ABRs threshold below 30 dB SPL were considered normal. One week after deafening, 70 dB SPL stimuli were applied. Animals that did not respond to the stimuli were re-tested; increasing the intensities by 10 dB steps. Animals with ABRs threshold greater than 90 dB SPL (corresponding to a threshold increase greater than 60 dB) were considered deaf and included in the present study.

### Preparation of cochlear specimens

All the animals, including the control group, were sacrificed 8 weeks after the beginning of the experiment. Rats were deeply anesthetized with diethyl ether. Cardiac perfusion was performed using 0.9% saline solution, followed by 10% formaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). After the temporal bones were removed, the cochleae were perfused through the round window with 2% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium phosphate buffer. They were immersed in the same fixative for 24 h. Decalcification was performed by means of 8.3% disodium ethylenediamine tetraacetic acid for 15 days, at 4°C. Decalcified cochleae were postfixed for 1 h in 1% osmium tetroxide in the same buffer. Specimens were dehydrated in acetone, cleared in propylene

oxide and embedded in Araldite resin. Serial semithin sections (1-3  $\mu\text{m}$ ) of each cochlea were obtained through the cochlear horizontal plane by means of an LKB III ultramicrotome, using glass knives. Sections were mounted on glass slides and stained with Stevenel's Blue. Five non-consecutive mid-modiolar sections of the cochlea were examined from each experimental animal.

### Morphology and Morphometry analysis of the SV

The SV at four different cochlear turns (apical, upper middle, lower middle and basal turns) were examined in five sections of each cochlea under a light microscope (X1000). All the photomicrographs were obtained using a Nikon digital camera and later assembled by means of the ArcSoft Panorama Maker 3.0 software. Brightness and contrast were adjusted in Adobe Photoshop 8.00.

Images of SV were acquired at X10 with a CCD camera connected to a personal computer and imported to the image analysis software Image J.<sup>16</sup> The SV area was outlined along the border of the marginal cells, from the spiral prominence to the Reissner's membrane, and laterally to the border of the strial basal cells and measured in  $\mu\text{m}^2$ . The number of capillaries per SV (NCSV), recognized by their lumen, was counted at X 1000.

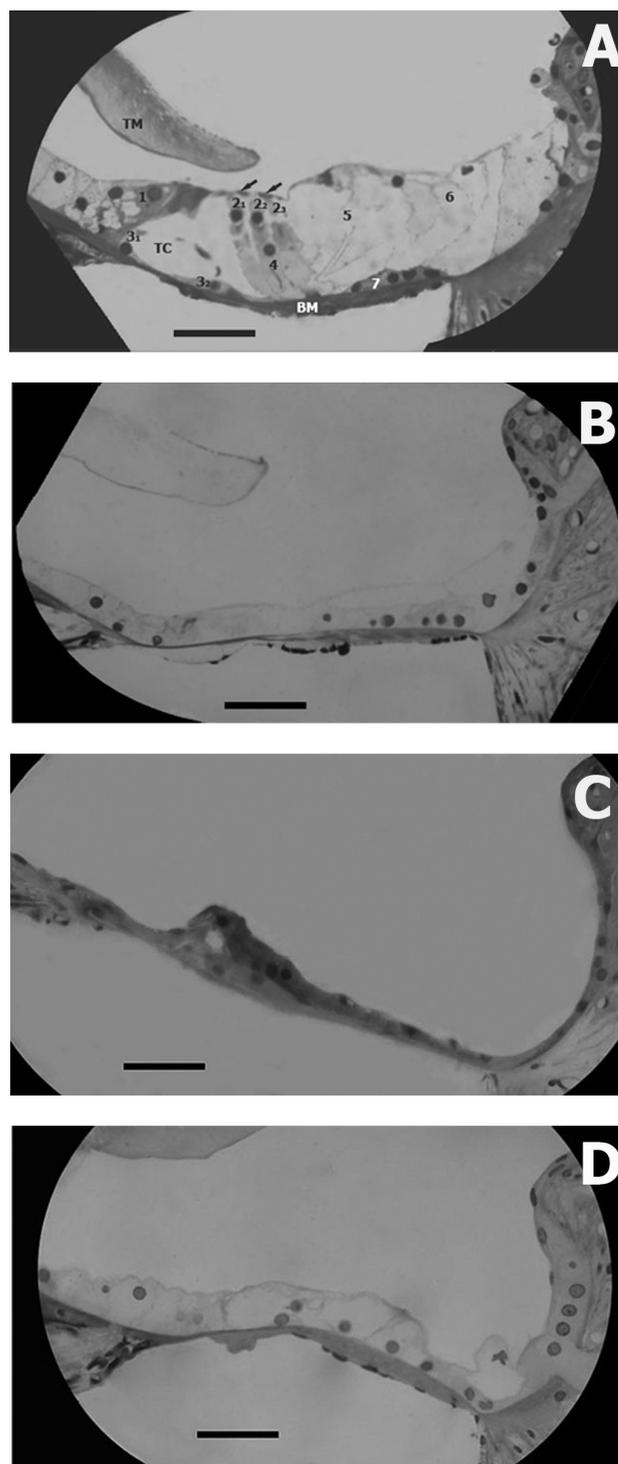
### Statistical analysis

The statistical analysis was undertaken using Statistica version 6.0 software. In order to evaluate the effects of the aminoglycosides. One-way ANOVA was carried out to compare the SV area of each cochlear turn among groups and the mean of the four cochlear turns among groups. ANOVA was always followed by the Tukey test. In the case of the variable NCSV, the four cochlear turns within each group and among animal groups were compared using the Kruskal-Wallis test, followed by the Test of multiple comparisons of mean ranks. Differences associated with  $p$  values  $< 0.05$  were considered to be significant. In addition, a Pearson correlation test was performed to explore the relationship between the SV area and the NCSV.

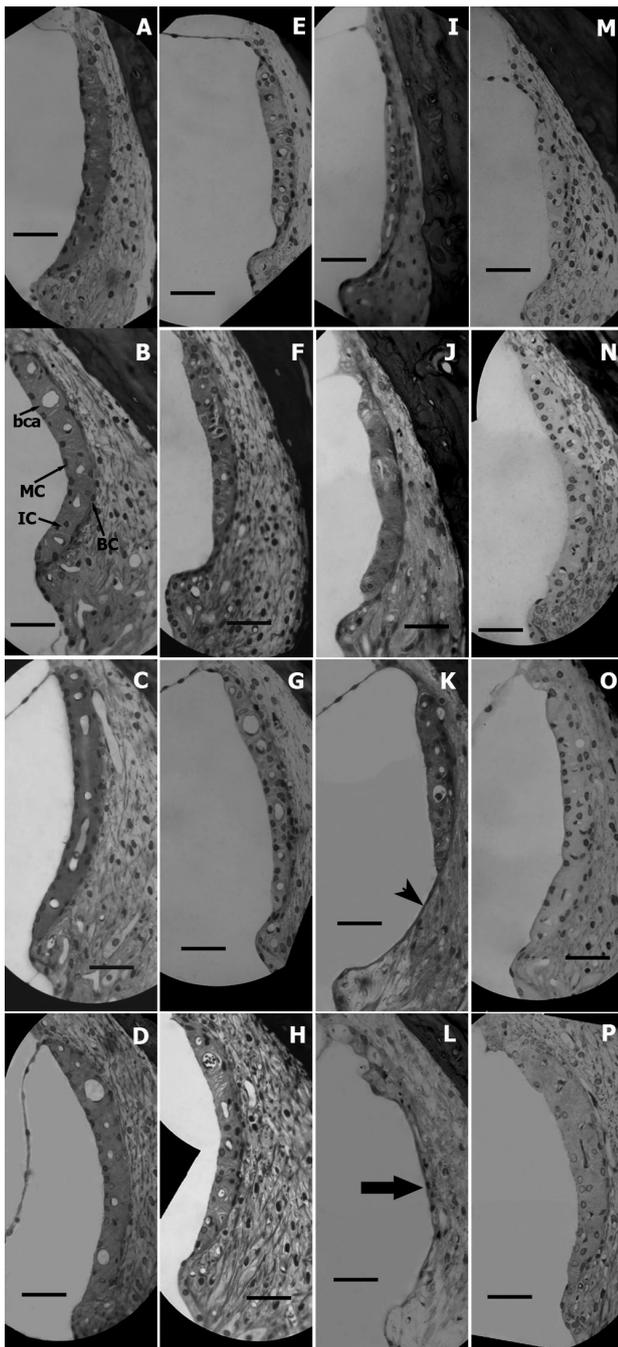
### Results

#### Morphology of the organ of Corti and the spiral ganglion

Normal hearing animals showed the normal organization pattern of the OC (Figure 1A). The OC of kanamycin and amikacin-deafened animals displayed a mosaic epithelium with a visible tunnel of Corti in the apical turn. Inner HC with well preserved nuclei were observed in some animals. However, various flattening degrees of the OC layer of cells were observed at the rest of the cochlear turns (Fig. 1B, D). No supporting cells could be morphologically identified in aminoglycoside-deafened groups, except for Boettcher's cells in the lower middle and basal turns in some animals. Among



**Figure 1.** Photomicrographs assembled by means of the ArcSoft Panorama Maker 3.00 of the organ of Corti (OC) at the lower middle cochlear turn. A: Control animal illustrating normal aspect of the OC characterized by the presence of: 1-inner hair cell; 21, 22, 23- outer hair cells (three rows); Arrows-cuticular plates; 31- inner pillar cell; 32- outer pillar cell; 4- Dieter cell; 5- Hensen cells; 6- Claudius cells; 7- Boettcher cells; TC-Tunnel of Corti; TM- Tectorial membrane; BM-Basilar membrane. B: Kanamycin-deafened animal illustrating partial flattening of the OC without hair cells and with evident Boettcher cells; C: Gentamicin-deafened animal illustrating total flattening of the OC without hair cells and supporting cells; D: Amikacin-deafened animal illustrating partial flattening of the OC without hair cells and with evident Boettcher cells (Stevenel's Blue; Scale bars = 50  $\mu\text{m}$ ).



**Figure 2.** Photomicrographs assembled by means of the ArcSoft Panorama Maker 3.00 of the stria vascularis (SV) along the four cochlear turns. A-D: Control animal illustrating normal SV structure at all cochlear turns. bca: blood capillaries, MC: marginal cells, IC: intermediate cells, BC: basal cells. E-P: Deafened animals. A, E, I, M: Apical; B, F, J, N: Upper middle; C, G, K, O: Lower middle; D, H, L, P: Basal. E-H: Kanamycin-deafened animal illustrating no visible changes in the stratified epithelium of the SV; I-L: Gentamicin-deafened animal illustrating from the end of the SV profile absent above the spiral prominence (arrowhead) to total flattening of the SV at the base (arrow); M-P: Amikacin-deafened animal illustrating lack of the capillary profiles (Stevens's Blue; Scale bars = 20  $\mu$ m).

the three aminoglycoside groups, gentamicin-deafened animals showed either a low cuboidal cell layer without recognizable supporting cells or a complete resorption of the OC in all cochlear turns (Fig. 1C).

On the other hand, all aminoglycoside-deafened animals showed abnormal morphology of SGN and their peripheral processes at all cochlear turns. The SGN density decreased in deafened animals at all cochlear turns, as compared to controls (results not shown).

#### *Morphology and morphometry of the stria vascularis*

For normal hearing animals, the SV was observed as a normal stratified epithelium integrated by basal, intermediate and marginal cells as well as blood capillaries (Figure 2A-D). These cells were arranged themselves as a row, starting from the Reissner's membrane towards the spiral prominence. In this group, both the SV area and the NCSV increased progressively from the cochlear apex to the basal turn (Figures 3 and 4, left panel).

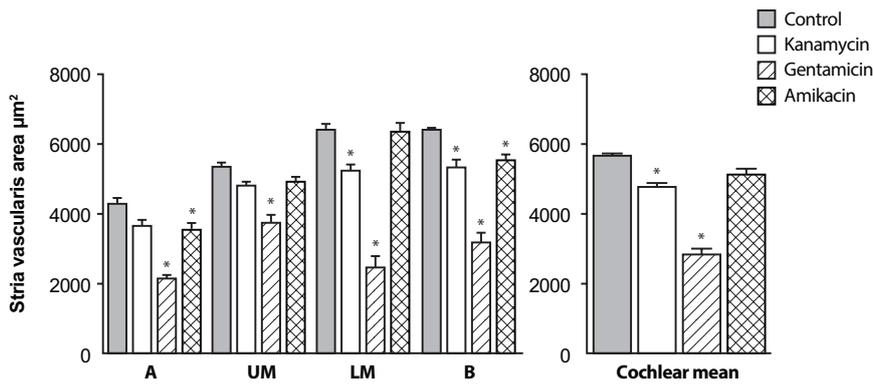
The normal SV area pattern along the cochlear turns was only observed in the kanamycin-deafened group. However, the end of SV profile was absent towards the spiral prominence, mainly in the apical and upper-middle turns (Figure 2E-H). As compared to controls, this group showed a SV area reduction in the lower middle and basal turns ( $F=49.43$ ,  $F=48.35$  respectively;  $p<0.001$ ) (Figure 3 left panel).

Gentamicin-deafened animals showed SV with several atrophy signs such as remnants of marginal cells without nuclei in the upper middle turn (Fig. 2J) and no cells in the lower middle turn (Fig. 2K). Similar to kanamycin-deafened animals, the end of the SV profile was absent towards the spiral prominence in the apical and upper-middle turns (Figure 2I-J). This was more remarkable in the lower middle turn (Fig. 2K). At the basal turn the SV disappeared leaving only a flat squamous epithelial layer (Fig. 2L). As compared to controls, this group showed reduced SV areas in all cochlear turns ( $F=75.11$ ;  $p<0.001$ ) (Fig. 3 left panel).

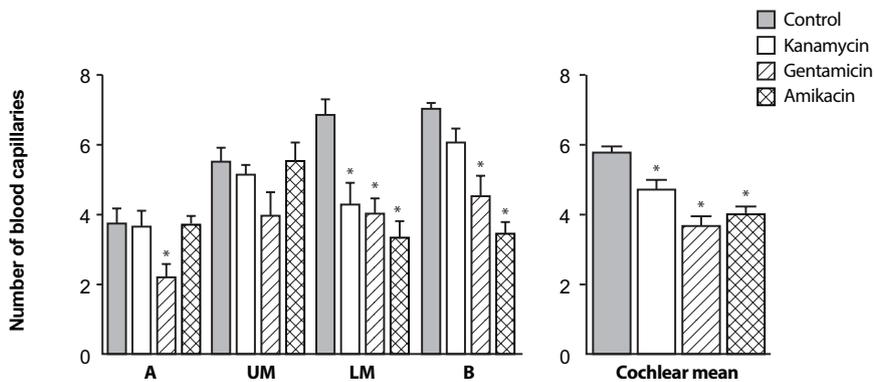
Amikacin-deafened animals showed the three SV cell types in every cochlear turn, except for the apical turn (Figure 2M-P), as well as a reduction in the SV area in the apical and basal turns ( $F=20.82$ ,  $F=48.35$ , respectively;  $p<0.001$ ) (Figure 3 left panel).

The mean SV area (calculated for the four cochlear turns) for amikacin-deafened animals was the same as the control values, although two of the four cochlear turns showed reduced SV areas. Mean SV area values for both kanamycin and gentamicin groups were lower than controls and the lowest values were observed for gentamicin-deafened animals (Figure 3 right panel).

The effects of the ototoxic treatments were also observed when the NCSV was analyzed, although the degeneration pattern was slightly different from the one of the SV area. As compared to the control group, kanamycin-deafened animals showed a reduced NCSV



**Figure 3.** Stria vascularis (SV) area at the four cochlear turns. Control and aminoglycoside-deafened groups. The plot illustrates the mean  $\pm$  standard deviation. Left panel: A: Apical turn, UM: Upper middle turn, LM: Lower middle turn, B: Basal turn. One-way ANOVA test, comparing the four groups. Asterisks indicate statistically significant differences compared to control group.  $p < 0.05$ , Tukey test, comparing between groups. Right panel: Mean SV area by groups. The SV area was more reduced for gentamicin than for kanamycin and amikacin-deafened animals.



**Figure 4.** Number of blood capillaries per stria vascularis (NCSV) for control and aminoglycoside-deafened groups along the cochlea. The plot illustrates the mean  $\pm$  standard deviation. Left panel: A: Apical turn, UM: Upper middle turn, LM: Lower middle turn, B: Basal turn. Kruskal-Wallis test, comparing the four groups. Asterisks indicate statistically significant differences compared to control group.  $p < 0.05$ , Test of multiple comparisons of mean ranks, comparing between groups. Right panel: Mean NCSV by groups. The NCSV was more reduced by gentamicin and amikacin than by kanamycin ( $p < 0.05$ ).

only in the lower middle turn of the cochlea ( $H=3$ ;  $p=0.005$ ) (Figure 4 left panel), while the NCSV of gentamicin-deafened animals was reduced in the apical ( $H=3$ ;  $p=0.018$ ), lower middle and basal turns ( $H=3$ ;  $p<0.0001$ ) (Figure 4 left panel). Amikacin-deafened animals showed a reduction of the NCSV in the lower middle and basal turns, as compared to controls ( $H=3$ ;  $p<0.0001$ ) (Figure 4 left panel).

Unlike the SV area, the mean NCSV was reduced for the three aminoglycoside-deafened groups with respect to control animals ( $H=3$ ;  $p<0.0001$ ) (Figure 4 right panel). The test of multiple comparisons of mean ranks showed that there were no differences between gentamicin and amikacin-deafened animals, and these values were lower than those obtained for the kanamycin-deafened group (Figure 4, right panel).

Correlation tests between SV area and NCSV revealed that only control and gentamicin-deafened animals showed positive correlation values ( $r=0.65$ ,  $p<0.0001$  and  $r=0.25$ ,  $p=0.023$ ; respectively).

## Discussion

Our results showed that the SV area increased from the cochlear apex towards its base in normal hearing animals. This result has been previously reported by other authors.<sup>12,17,18</sup> The cochlear damage degree depended on the aminoglycoside. Reductions of mean SV areas induced by kanamycin and gentamicin eight weeks after deafness were shown in

this work. Hellier et al.<sup>12</sup> reported that the degree of kanamycin-induced SV degeneration in the guinea pig model was directly dependent on the duration of the hearing loss. They found that 16 weeks after deafness, the SV degeneration extended to all cochlear turns. Similar results were found in another work using kanamycin and bumetanide in a mouse model. Remarkable SV degeneration with loss of the normal cytoarchitecture and reduction of the SV thickness to about a third, was described 8-16 weeks after treatments.<sup>13</sup> Similar results were obtained using repeated doses of kanamycin and furosemide in a different mice strain as early as 2 weeks after the end of the treatments.<sup>19</sup>

On the other hand, loop diuretics, are known to cause short term changes in the morphology of the SV, but permanent changes have not been observed.<sup>20</sup>

The three aminoglycosides used in the present work induced a severe reduction in NCSV as compared to controls. It has been reported that aging, a known cause of deafness, induces reduction of capillary diameters or produces avascular SV. However, no previous references were found regarding aminoglycoside effects upon the NCSV.<sup>21</sup>

Like the SV area, the NCSV of normal hearing animals increased from the cochlear apex to the base. It has been suggested that a wider SV at the cochlear base is characteristic of a more organized pattern for the arterioles, which assures a better communication between arteries and veins.<sup>21</sup> For control animals, a positive correlation between the SV area and the NCSV was found.

This positive correlation was also observed for gentamicin-deafened animals. In 1995, Gratton et al.<sup>22</sup> suggested that regions of SV atrophy in aged gerbils usually lacked capillaries. Gratton et al.<sup>23</sup> and Ohlemiller et al.<sup>24</sup> suggested that atrophy of the SV in aged animals is a secondary pathological event that occurs in response to partial or complete vascular occlusion. No correlation was found between SV area and NCSV in amikacin-deafened animals. A possible reason for this result could be that while the NCSV of amikacin-deafened animals was reduced as compared to controls, the remaining SV marginal, intermediate and basal preserved cells were responsible for the maintenance of the SV area.

The mechanism by which aminoglycosides generate deafness is still a subject of debate. Many of the adverse effects have been attributed to a specific interaction with cell membrane phospholipids, especially with the polyphosphoinositides present in both the OC and the SV. It has been shown that aminoglycosides alter the physical state of cell membrane lipids. Forge and Fradis<sup>19</sup> observed that gentamicin causes morphological changes on liposomes, which also contain polyphosphoinositides and other anionic phospholipids. On the other hand, Hellier et al.<sup>12</sup> suggested that the SV degenerative changes induced by kanamycin in guinea pigs were due to a reduced metabolic burden following the loss of the OC, rather than to a direct intoxication. Other authors, after finding a gradual capillary loss in the spiral ligament of aged humans, postulated that the SV is essential for the development of the OC and suggested that the SV dysfunction preceded the OC degeneration.<sup>25</sup>

Recent theories ascribe the cochlear damage induced by aminoglycosides, noise exposure and aging, to oxidative stress mechanisms. Choung et al.<sup>26</sup> detected highly-reactive oxygen species in explants of the OC after gentamicin exposure and suggested that their accumulation is an important initial step in hair cell damage. The differential sensitivity of hair cells is closely related to differences in highly-reactive oxygen species accumulation.

Although the action mechanisms of ototoxic agents in the cochlea need to be studied in more detail, the diverse effects shown in this work could be related to the different extent of the oxidative stress caused by the three aminoglycosides. This idea arises from experiments that analyzed the generation of free-radicals in cochlear explants treated with different aminoglycosides. Clerici et al.<sup>27</sup> demonstrated that gentamicin induces the formation of more reactive oxygen species than other ototoxic agents such as kanamycin, different diuretics and cisplatin.

The effects of different aminoglycosides on the cochlear morphology have been studied by other authors both *in vitro*<sup>28</sup> and *in vivo*.<sup>29,30</sup> In accordance to our results, these previous studies found gentamicin to be the most aggressive ototoxic aminoglycoside. However, they focused on the effect of the ototoxic treatment on the HC, and no comparative reports were found for the SV.

## Conclusion

The present results demonstrated that the magnitude of the SV degeneration caused by the ototoxic treatments depended on the aminoglycoside used. For both the SV area and NCSV, the gentamicin-deafened animals showed the most severe damage.

## Acknowledgements

The authors thank to Teresa Diez for animal maintenance and to Yasser Iturria for his assistance and suggestions during statistical analysis of our results. We would also like to thank the staff of the Pathology Department of the Institute of Neurology and Neurosurgery where microscopic observations and image acquisition for morphometry were made.

## References

1. Bodmer D. Protection, regeneration and replacement of hair cells in the cochlea: implications for the future treatment of sensorineural hearing loss. *Swiss Med Wkly* 2008; 138: 708-712.
2. Corbacella E, Lanzoni I, Ding D, Previati M, Salvi R. Minocycline attenuates gentamicin induced hair cell loss in neonatal cochlear cultures. *Hear Res* 2004; 197: 11-18.
3. Pirvola U, Xing-Qun L, Virkkala J, Saarma M, Murakata C, Camoratto AM, et al. Rescue of hearing, auditory hair cells, and neurons by CEP-1347/KT7515, an inhibitor of c-Jun N-terminal kinase activation. *J Neurosci* 2000; 20: 43-50.
4. Dodson HC. Loss and survival of spiral ganglion neurons in the guinea pig after intracochlear perfusion with aminoglycosides. *J Neurocytol* 1997; 26: 541-556.
5. Sugawara M, Corfas G, Liberman MC. Influence of supporting cells on neuronal degeneration after hair cell loss. *J Assoc Res Otolaryngol* 2005; 6: 136-147.
6. McFadden SL, Ding D, Jiang H, Salvi RJ. Time course of efferent fiber and spiral ganglion cell degeneration following complete hair cell loss in the chinchilla. *Brain Res* 2004; 997: 40-51.
7. Shepherd RK, Coco A, Epp SB, Crook JM. Chronic depolarization enhances the trophic effects of brain-derived neurotrophic factor in rescuing auditory neurons following a sensorineural hearing loss. *J Comp Neurol* 2005; 486: 145-158.
8. Murillo-Cuesta S, Contreras J, Cediell R, Varela-Nieto I. Comparison of different aminoglycoside antibiotic treatments to refine ototoxicity studies in adult mice. *Lab Anim* 2009; 44: 124-131.
9. Sha SH, Taylor R, Forge A, Schacht J. Differential vulnerability of basal and apical hair cells is based on intrinsic susceptibility to free radicals. *Hear Res* 2001; 155: 1-8.
10. Wangemann P. K<sup>+</sup> cycling and the endocochlear potential. *Hear Res* 2002; 165: 1-9.
11. Wangemann P. Supporting sensory transduction: cochlear fluid homeostasis and the endocochlear potential. *J Physiol* 2006; 576: 11-21.

12. Hellier WP, Wagstaff SA, O'Leary SJ, Shepherd RK. Functional and morphological response of the stria vascularis following a sensorineural hearing loss. *Hear Res* 2002; 172: 127-136.
13. Taylor RR, Nevill G, Forge A. Rapid hair cell loss: a mouse model for cochlear lesions. *J Assoc Res Otolaryngol* 2008; 9: 44-64.
14. Carlisle L, Forge A. The vessels of the stria vascularis: quantitative comparison of three rodent species. *Hear Res* 1989; 38: 111-117.
15. Alam SA, Ikeda K, Kawase T, Kikuchi T, Katori Y, Watanabe K, et al. Acute effects of combined administration of kanamycin and furosemide on the stria vascularis studied by distortion product otoacoustic emission and transmission electron microscopy. *Tohoku J Exp Med* 1998; 186: 79-86.
16. Rasband W. ImageJ: A public domain Java image processing program. National Institute of Mental Health, Bethesda, Maryland, USA 2008 Available from: URL: <http://rsb.info.nih.gov/ij/>
17. Santi PA, Muchow DC. Morphometry of the chinchilla organ of Corti and stria vascularis. *J Histochem Cytochem* 1979; 27: 1539-1542.
18. Lohuis PJ, Patterson K, Rarey KE. Quantitative assessment of the rat stria vascularis. *Hear Res* 1990; 47: 95-102.
19. Hirose K, Sato E. Comparative analysis of combination kanamycin-furosemide versus kanamycin alone in the mouse cochlea. *Hear Res* 1985; 20: 233-244.
20. Rybak LP, Whitworth C, Weberg A, Scott V. Effects of organic acids on the edema of the stria vascularis induced by furosemide. *Hear Res* 1992; 59: 75-84.
21. Lopes AL, Costa CA. Structure and Ultrastructure of the Mammalian Inner Ear with Emphasis in the Cochlea. *Int Arch Otorhinolaryngol* 2006; 10: 228-240.
22. Gratton MA, Schulte BA. Alterations in microvasculature are associated with atrophy of the stria vascularis in quiet-aged gerbils. *Hear Res* 1995; 82: 44-52.
23. Gratton MA, Schmiedt RA, Schulte BA. Age-related decreases in endocochlear potential are associated with vascular abnormalities in the stria vascularis. *Hear Res* 1996; 102: 181-190.
24. Ohlemiller KK, Rice ME, Gagnon PM. Strial microvascular pathology and age-associated endocochlear potential decline in NOD congenic mice. *Hear Res* 2008; 244: 85-97.
25. Johnsson LG, Hawkins JE, Jr. Strial atrophy in clinical and experimental deafness. *Laryngoscope* 1972; 82: 1105-1125.
26. Choung YH, Taura A, Pak K, Choi SJ, Masuda M, Ryan AF. Generation of highly-reactive oxygen species is closely related to hair cell damage in rat organ of Corti treated with gentamicin. *Neuroscience* 2009; 161: 214-26.
27. Clerici WJ, Hensley K, DiMartino DL, Butterfield DA. Direct detection of ototoxicant-induced reactive oxygen species generation in cochlear explants. *Hear Res* 1996; 98: 116-124.
28. Kotecha B, Richardson GP. Ototoxicity in vitro: effects of neomycin, gentamicin, dihydrostreptomycin, amikacin, spectinomycin, neamine, spermine and poly-L-lysine. *Hear Res* 1994; 73: 173-184.
29. Kitasato I, Yokota M, Inouye S, Igarashi M. Comparative ototoxicity of ribostamycin, dactimicin, dibekacin, kanamycin, amikacin, tobramycin, gentamicin, sisomicin and netilmicin in the inner ear of guinea pigs. *Chemotherapy* 1990; 36: 155-68.
30. Kalkandelen S, Selimoglu E, Erdogan F, Ucuncu H, Altas E. Comparative cochlear toxicities of streptomycin, gentamicin, amikacin and netilmicin in guinea-pigs. *J Int Med Res* 2002; 30: 406-412.