Resumen

La sordera es una de las discapacidades mundiales más comunes. Su causa más frecuente es la muerte de las células ciliadas cocleares (localizadas en el órgano de Corti), lo que induce la degeneración de las neuronas del ganglio espiral y sus procesos periféricos que inervan el órgano de Corti. En un estudio previo por microscopía óptica, usando un modelo de ototoxicidad en ratas, se observó la pérdida de las neuronas del ganglio espiral desde la octava semana de sordera y la degeneración de los procesos periféricos desde la cuarta semana. Con el objetivo de determinar el comienzo de los cambios ultraestructurales degenerativos se llevó a cabo un estudio por microscopía electrónica de transmisión de las neuronas del ganglio espiral y sus procesos periféricos. Se analizaron cócleas de ratas tomadas después de 2, 4, 8 y 16 semanas de sordera y controles sanos. Las neuronas tipo I del ganglio espiral y las bandas de mielina de sus procesos periféricos mostraron cambios degenerativos progresivos desde la cuarta semana de sordera. La mayoría de las neuronas que quedaron exhibieron una completa desmielinización a las 16 semanas de sordera, resultando en las neuronas patológicas del ganglio espiral tipo III. Estos resultados evidencian los cambios ultraestructurales degenerativos de las neuronas del ganglio espiral y sus procesos periféricos, antes de que ambos sufran pérdidas significativas.

Palabras claves: Cóclea, Ototoxicidad, Neuronas del ganglio espiral, Procesos periféricos, Microscopia electrónica de transmisión.

Abstract

Deafness is one of the most widespread disabilities in the world. Its most frequent cause is death of cochlear hair cells (located in the organ of Corti), which induces degeneration of spiral ganglion neurons and their peripheral processes innervating the organ of Corti. In a previous light microscopy study using a rat model of ototoxicity, a loss of spiral ganglion neurons was observed since the eighth week of deafness and peripheral processes degeneration since the fourth week. In order to determine the onset of ultrastructural degenerative changes, a transmission electron microscopy study of spiral ganglion neurons and their peripheral processes was undertaken. Rat cochleae sampled after 2, 4, 8 and 16 weeks of deafness and healthy controls were analyzed. Since the fourth week of deafness, Type I spiral ganglion neurons and the myelin sheaths of their peripheral processes showed progressive degenerative changes. Most of the remaining neurons exhibited complete demyelination at sixteen weeks of deafness, resulting in the pathological type III spiral ganglion neurons. These results show ultrastructural degenerative changes of the spiral ganglion neurons and their peripheral processes, before both undergo significant losses.

Key words: Cochleae, Ototoxicity, Spiral ganglion neurons, Peripheral processes, Transmission electron microscopy.

Introduction

Deafness is one of the most common disabilities in the world.1 Its incidence in Cuba is 2.1 per 1,000 inhabitants.2 The primary cause of human auditory loss is the alteration or death of sensorial receptor cells of the inner ear, the cochlear hair cells, located in the organ of Corti (OC).3 Mammalian hair cells cannot regenerate after damage,4 their loss lead to permanent sensorineural deafness, which is characterized by loss of hair cells and retrograde degeneration of the spiral ganglion (SG) neurons.

Cochlear implants are crucial to recover the audition for patients with sensorineural deafness.5 Their function is based on the stimulation of the axons of the SG type I neurons.6 Effective cochlear implants which may work with only 10% of the original population of SG neurons have been developed.7 Moreover,
the preservation degree of SG neurons is considered decisive for language recognition after a cochlear implant. SG neuronal degeneration could be a restrictive factor against the optimal function of more advanced cochlear implants.

The cochlear implant performance is also influenced by the loss of the peripheral processes innervating the OC because the action potentials generation site needs to be transferred either towards the soma of the SG neurons or toward the central axon.

The potential application of therapies based on drugs or on stem cells to protect or to regenerate SG neurons as an alternative to overcome deafness, has motivated recent interest in studying the ultrastructure of these neurons.

In adult rats, a decrease of the SG neuronal density starting from the 8th week of deafness, as well as the emptying of the osseous spiral lamina due to the loss of the peripheral processes innervating the OC since the 4th week, have been shown by light microscopy in a previous paper. The aim of the present work was to study the ultrastructure of the SG neurons and their peripheral processes in order to determine the onset of the ototoxicity-induced degenerative changes.

Materials and methods

Animals: Adult male Wistar rats weighting from 250 to 300 g were provided by the National Center for Laboratory Animal Production. At the beginning of the experiment, the normal function of the auditory pathway was confirmed in all the animals by means of brainstem auditory evoked potentials (BSAEP) and steady state auditory evoked potentials (SSAEP).

Animals were divided into five groups with two rats on each: control (non-treated rats) and four groups of rats treated with the ototoxic agents. The latter were sacrificed after 2, 4, 8 and 16 weeks of deafness.

Deafness induction: At the beginning of the experiment, the four treated groups simultaneously received intraperitoneal kanamycine (400 mg/kg) and furosemide (150 mg/kg). One week later, hearing loss was confirmed by means of BSAEP and SSAEP. Those animals that didn’t answer to 105 dB pspl intensity for BSAEP or to 105 dB spl for SSAEP, were considered deaf.

Sample processing: The animals were fixed by vascular perfusion in 10% formalin. A cochlea was removed from each rat and immediately fixed by perilymphatic perfusion in 2% paraformaldehyde and 2% glutaraldehyde (in 0.1 mol/L, pH 7.4 sodium phosphate buffer). The cochleae were decalcified in 8,3% EDTA, post-fixed in 1% osmium tetroxide (in the same buffer), dehydrated in acetone and embedded in Spurr resin. The samples were cut through the cochlear horizontal plane with an Ultrotome III (LKB) ultramicrotome. Semithin cochlear sections dyed with Stevenel Blue and mounted on glass slides were obtained to locate the medial cochlear turn by light microscopy. ultrathin sections of the cochlear medial turn were placed on 400 mesh grids, stained with uranyl acetate and lead citrate and examined under a Transmission Electron Microscope Jeol JEM 100S.

Results

Spiral ganglion neurons

Control cochleae SG neurons showed intact myelin sheaths, round nuclei, several narrow channels of rough endoplasmic reticulum (Nissl bodies) and mitochondria with normal cristae (Fig. 1A).

Since the fourth week of deafness, prominent irregularities in both the myelin sheath and the nuclear envelope were observed (Fig. 1B) together with cytoplasmic matrix clearing: scarce Nissl bodies (Fig. 1C).

After eight weeks of deafness, the remaining Type I SG neurons showed cytoplasmic shrinkage related to their myelin sheaths and conspicuous cytoplasmic inclusions (Fig. 1D). Nuclear envelope invaginations, dilated Nissl bodies and cytoplasmic vacuolization were also evident (Fig. 1E).

Sixteen weeks after deafness, most of the remaining Type I SG neurons exhibited complete demyelination, which resulted in the pathological Type III SG neurons with dense mitochondria and scarce Nissl bodies (Fig. 1F).

Peripheral processes
In normal cochleae, the myelinated peripheral processes showed intact myelin sheaths and extensive Schwann cell cytoplasm surrounding them (Fig. 2A). In deafened cochleae, myelin sheaths and Schwann cells cytoplasm were altered. Four weeks after deafness, zones of separation between the layers of the myelin sheaths and rupture points of the sheaths were observed (Fig. 2B). Eight and sixteen weeks after deafness, myelin sheaths discontinuities with axoplasm efflux were observed (Fig. 2C). The density of the peripheral processes suffered a progressive reduction since the fourth week of deafness (Fig. 2B-D).

Fig 1. Ultrastructure of SG cells. A) Control. B-F) Deafened cochleae. B, C) 4 weeks, D, E) 8 weeks, F) 16 weeks. Myelin sheath (arrows), nucleus (N), mitochondria (M), Nissl bodies (Nb), Golgi vesicles (Gv), cytoplasmic retraction from the myelin sheath (CR), cytoplasmic inclusions (small arrows), vacuoles (V).
Fig. 2. Ultrastructure of peripheral processes innervating the organ of Corti. A) Control, B-D) Deafened rats, B) 4 weeks, C) 8 weeks, D) 16 weeks. Myelin sheaths (arrows), Schwann cells (*).

Discussion

It has been proved that the aminoglycoside kanamycin induces sensorineural deafness, in which the hair cells loss precedes the degeneration of SG neurons. Furosemide induces clinically relevant transitory ototoxic damage and, as other diuretics, it enhances the ototoxicity of aminoglycosides. The medial cochlear turn was chosen for the electron microscopical observations because it showed the greatest neuronal loss in deaf animals related to controls. SG neurons convey information from the cochlea to the central auditory system. In normal cochleae, two types of SG neurons (Type I and Type II) have been described within the Rosenthal's canal. The present work focused its attention on type I SG neurons, since they represent approximately 95% of the SG neurons in rat cochleae. Besides, they are more susceptible to injury than type II and are considered of primary relevance in the application of cochlear implants.

Degenerative changes on SG neurons were ultrastructurally detected in this work 4 weeks before their loss was revealed by light microscopy. Progressive degenerative changes of type I SG neurons after deafness result in the emergence of type III neurons, exclusive of pathological conditions.

In humans, most of the hearing losses are sensorineural and it is considered that SG neuronal damage is due to the failure of the trophic effect exerted by hair cells over them. By light microscopy, the scarcity of peripheral processes innervating the OC together with the loss of SG neuronal somata has been demonstrated in deaf animals. In this paper, by electron microscopy, it was shown that the remaining peripheral processes undergo progressive degeneration since the fourth week of deafness. The condition of the OC supporting cells also influences upon SG neuronal survival. In previous works, degeneration of the OC hair cells and supporting cells was observed since the second week of deafness.

Conclusions

In this work, degenerative changes on SG neurons were ultrastructurally detected 4 weeks before the loss of SG cells and their peripheral processes were revealed by light microscopy. Provided the similarities between the inner ear of rodents and humans, the information from these animal models of deafness might be taken into account when considering cochlear implants to patients affected by ototoxic drugs.

Although cochlear implants are at present the best option available for deafness treatment, they do not completely restore the auditive function. Prevention of hair cell death or their regeneration, as well as regeneration of the SG neurons to innervate the new hair cells, are among the new therapeutical
strategies that are being studied. The latter approaches demonstrate the importance of understanding the morphological changes that occur in the cochlea submitted to ototoxicity.

References


