Introduction

The mammalian utricular macula as part of the vestibular system is a shell shaped organ covered with sensory epithelium. The sensory epithelium of the utricle is composed of type I cells (large), type II cells (small), and supporting cells which generally separate and surround the sensory cells. The upper surface of the type I and type II cells is covered by 20 to 100 stereocilia and a single kinocilium. The stereocilia are arranged in a step–like fashion, according to their length. The longest stereocilium is positioned adjacent to the kinocilium. The cilia of the sensory cells change their polarization along a narrow curved zone extending through the middle of the macula. This area is known as the striola. The distribution of these two types of sensory cells seems to be rather random; however type I cells are more highly concentrated in the striolar area than in the periphery and the type II cells are more concentrated in the inner zone of the utricle. The type I cells are flask–shaped, while the type II cells own a cylindrical shape. The type I cell is surrounded by an unmyelinated nerve chalice, which is the ending of large myelinated afferent nerve fiber. The type II cells make contact with both efferent and afferent nerve fibers\(^{(1–3)}\).

It is well known that aminoglycosides induce hair cell degeneration in the inner ear. This degeneration is described to be apoptotic\(^{(4,5)}\). Systemic administration\(^{(6,7)}\) of aminoglycosides appears to be less destructive than intraotic application\(^{(6,7)}\) and a difference in susceptibility is found between different parts and cell types in the inner ear\(^{(5–9)}\). No inflammation is observed during the destruction of the sensory epithelium\(^{(4,7–10)}\). The striolar region of the utricle is found to be more sensitive to aminoglycosides than the...
peripheral areas, which indicates a higher susceptibility of type I sensory cells for aminoglycoside ototoxicity.

Hair cell regeneration in frogs and birds after aminoglycoside–treatment is reported in several experimental studies \((11–13)\). In two subsequent studies Forge reported the presence of small hair bundles on cells in the utricle of mammals, appearing four weeks after ending gentamicin application \((4,6)\). The apical surfaces of these cells were smaller than those of mature hair cells, however they showed distinct, organized bundles of small stereocilia. The length of all stereocilia was equal and in their center a longer and thicker kinocilium was observed. In non–treated utricles immature hair cells were found as well, however the number of immature hair bundles was substantially larger in treated utricles. More recently, Walsh reported morphological evidence for significant regeneration \((66\%)\) of mammalian utricular sensory epithelium, ten months after gentamicin–induced damage \((14)\).

In this experimental study a scanning electron microscopic investigation of the utricular sensory cells after systemic application of aminoglycosides was performed with emphasis on the histo–pathological repair mechanisms of the vestibular sensory epithelium.

Materials and methods

Eleven healthy female albino guinea pigs (Harlan, the Netherlands), with a positive Preyer’s reflex and a weight of 400–500 gram were used in this experiment. The experimental Animal Committee of the Groningen University approved animal care and use, in accordance with the principles of the Declaration of Helsinki (protocol number 2325).

Administration of gentamicin

Intra–muscular injections were given daily, during three periods of five days, with a pause of two days between the periods. One group \((n=7)\) received 100mg/kg and the other group \((n=4)\) was used as a control group. One week after the last administration of gentamicin the guinea pigs were sacrificed by means of an intra–cardial injection of sodium pentobarbital \((60\ \text{mg/kg})\) for SEM investigation of the utricles.
Fixation

After termination, the animals were decapitated; the temporal bones were removed and placed in ice-cold HBSS (pH 7.4; 320 Mosm; 0–4°C). The vestibulum was opened, and the utricle was located and isolated. With a fine pair of tweezers the otolithic membrane was gently removed and then the utricle was fixated in a solution of 2.5% glutaraldehyde in 0.1M Na–cacodylate buffer (pH 7.4; 400 Mosm; 4°C) and 2mM calcium chloride.

Scanning Electron Microscopy

The TAO–method was performed as post–fixation (15). This method uses a combination of tannic acid, arginine hydrochloride, glycine, sodium glutamate, sucrose and OsO4 for optimal preservation and contrast of ultrastructures.

After postfixation, the specimens were rinsed in distilled water and dehydrated in ethanol. All specimens were Critical Point dried with liquid CO2 and were sputtercoated with Au–Pd (10nm) according to routine procedures. The specimens were studied in a JEOL FEG–SEM, type 6301F, operating at 5kV.
Figure 1a shows a shell shaped utricle covered by sensory epithelium. The outlined area is the gentamicin–affected region. The affected area is situated in and next to the striola. Hardly any damage is observed in the peripheral regions.

Figure 1b (detail from figure 1a) clearly displays hair cell loss and holes (arrows) in the striolar region of the utricle.
Figure 2a displays fusion of stereocilia (F), hair cells with a kinocilium (K) and a reduced number of stereocilia. In one hole (arrow) replacement by matrix–material can be observed while the other (arrowhead) shows the gap after rejection of a hair cell.

Figure 2b demonstrates a hair cell bundle with fusion (F) during rejection from the utricular epithelium (arrows).
Results

The control group showed the specific surface morphology of utricular sensory epithelium without pathological changes. The type I and type II hair cells demonstrated a characteristic step-like arrangement of the stereocilia and the usual configuration of the kinocilium in relation to the stereocilia. No signs of post-fixation artefacts were observed.

In all specimens treated with gentamicin, progressive degeneration and loss of hair cells were observed alongside the striolar region (figure 1A, B) and in some cases the peripheral zones were affected as well. The density of the hair bundles in the peripheral areas seemed to be normal.

At higher magnification the remaining hair bundles in the affected areas showed a degenerative pattern which consisted of fused and decreased numbers of stereocilia, dissociation of hair bundles, lifting or sinking of the cuticular plate and rejection of complete hair cells (figure 2A, B). In the severely damaged striolar areas holes were found in the epithelium of all specimens (figure 2A).

Increased numbers of microvilli with extended length were detected in the affected areas. It was not possible to identify the cell type (I or II) of the degenerative cells. A limited number of relatively undamaged sensory cells in the striolar region could be characterized as type II hair cells (small hair bundles with stepped stereocilia and a long kinocilium). The number of supporting cells had increased in the affected areas in comparison with the control group.

In some specimens hair bundles could be observed in the striolar region next to severely damaged sensory hair cells. These hair bundles consisted of short stereocilia of equal height angled toward the cell center, in which a short kinocilium is located (figure 3).

Discussion

This study demonstrates the morphological damage to the utricle after systemic application of gentamicin. In the control group a normal morphology was observed, which excludes artefacts as a possible explanation for the described alterations. Comparable damage of the utricle after systemic administration of gentamicin is described by Wersäll et al. (9), Twine (8) and Forge (6,16). It is well known that topical application of gentamicin near the round window (7,10,16) in comparison with systemic application, provides a quicker and more devastating effect on all parts of the inner ear.
Figure 3  Different stages of development of immature hair cells. Figure 3A shows microvilli (M) with a pattern which is characteristic for an immature hair cell. A kinocilium is not observed here. Figure 3B displays a hair bundle with stereocilia (S) nearly as thick as the surrounding microvilli. A kinocilium is found here (arrow). Figure 3C and 3D demonstrate a clear view of young hair cells with stereocilia (S), which can be distinguished from the surrounding microvilli and a more obvious kinocilium (arrow) is displayed. Bar = 2 µm.
Li et al. (5) described two modes of hair cell loss in the vestibular sensory epithelium of the guinea pig: Degeneration of hair cells within the epithelium (incorporation of cell fragments into adjacent undamaged cells) and rejection of hair cells, followed by replacement with adjacent supporting cells. In all gentamicin–treated specimens of our experiment we observed holes in the striolar region, suggesting a process of rejection of sensory cells. The inferior and lateral surroundings of these holes could clearly be identified and may indicate the ingrowths of supporting cells.

Scanning microscopic evaluation was performed in our experiment after three weeks of intramuscular gentamicin administration followed by a recovery period of one week. Since several other studies in mammals, birds and frogs (6, 11, 13, 14-16, 17, 18) only showed hair cell regeneration in the vestibular system after a recovery period of at least four weeks, signs of regeneration were not expected in our study. However, in the utricle immature hair cells were observed in severely damaged striolar regions. The origin of these cells is unclear. Immature hair cells can be found in the normal macula utriculi (19) although in very limited numbers (0.7%). In the severely damaged areas of the utriculi we found small hair cell bundles, which might indicate that regeneration of hair cells in mammals is initiated earlier than previous studies have shown. However, most previous studies used topical application of aminoglycosides, which has a fast and more devastating effect on the inner ear and vestibular system. Another explanation is that immature hair bundles of the vestibular system are more resistant to gentamicin than mature hair cells.

Future studies on function and submicroscopical morphology are needed to provide more evidence regarding the regenerative potential of the vestibular sensory epithelium after aminoglycoside ototoxicity.
Histopathology of vestibular sensory epithelium after gentamicin induced injury in the guinea pig.