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Dose Dependent Changes In The Crista Neuroepitheium Resulting From Gentamicin Ototoxicity In The Chinchilla

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Abstract: This experiment was conducted to investigate the dose-dependent effects of gentamicin (GM) on the neuroepithelial morphology of the posterior crista ampullares (PCA) of the adult chinchilla.

The experiment focused on hair cell (HC) morphology to monitor GM's dose-dependent effects and also to define an ototoxically-efficient and safe dose what would subsequently be used in a time course study.

Chinchillas (n=13) were treated with subcutaneous (SC) daily doses of 120 mg/kg, 60 mg/kg, and 30 mg/kg GM, respectively, for six days. Light microscopy observations were made from the excised PCA of two animals from each group that survided the complete treatment regimen plus one day post-treatment (PT); these were compared to an untreated control group (n=4). Nephrotoxicity of GM was established by blood urea nitrogen (BUN) and creatinine measurements. The 120 and 60 mg/kg doses yielded a higher deterioration of kidney function with elevated levels of BUN and creatinine; pathological morphology within the kidneys was also observed. These findings were incompatible with the survival of the animals. In addition, results from one day post-treatment at each of the three different doses revealed non-specific morphological changes such as fusion of the hairs of the cells, shrunken cytoplasm and blebs. These results led us to choose the lower, safer, yet ototoxically-efficient dose of 30 mg/kg to study the time course of the GM-induced effects in a second experiment.

Key Words: Hair cells, gentamicin ototoxicity, nephrotoxicity, crista ampullaris, chinchilla

Introduction

Historically, gentamicin (GM), an ototoxic aminoglycoside antibiotic with high vestibular selectivity, has been used in vestibular research mainly to induce morphological change via its cochleo-vestibulotoxic effects (1, 2, 3, 4, 5). The vestibulotoxic effect of GM on hair cells proceeds through a cascade of irreversible, biochemically disruptive events which may ultimately result in death of these cells (6, 7). Interestingly, GM also affects the proximal tubular cells in the kidney through the same biochemical pathways, but the damage in this case is temporary and is reversed through regeneration of new epithelial cells over time (8, 9). We designed a study to define a safe and efficient ototoxic dose to evaluate the changes in the vestibular system hair cells and kidney tubular cells of the chinchilla mammalian model. GM was systemically administered to induce hair cell pathology in the crista neuroepithelia (NE) of the chinchilla in order to evaluate the dose-dependent changes. In addition, histological kidney data documented neprotoxicity supported definition of a efficient and safe otoxic dose for further use in an another experiment. The specific question that was addressed was: What are the changes in the crista NE after different doses of GM?

Material and Methods

Adult chinchillas (*Chinchilla Laniger*), 8 to 12 months of age, weighing 450-550 grams were utilized. Three groups of animals (n=13) were treated with subcutaneous (SC) daily doses of 120 mg/kg (n=5), 60 mg/kg (n=5), and 30 mg/kg (n=3), GM, respectively, for six days. The animals were sacrificed on the first day after the treatment. Observations were made from the PCA of two animals from each group that survived the complete treatment regimen plus one day post-treatment (PT). One

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of the objectives of this experiment was to determine a GM dose that could be tolerated by the animals and thereby effectively used to further study GM-induced NE pathology. To assist in this objective, GM's well-known nephrotoxic effects were traced through measures of blood urea nitrogen (BUN) and creatinine levels that were determined from blood samples drawn at the time of perfusion (n=21). Microscopic pathology of the kidneys was also assessed.

Morphological Analysis

At the time of sacrifice for the surgical extraction of the respective PCAs, animals were deeply anesthetized (IM administration of a ketamine/xylazine mixture, 20 mg/kg & 3 mg/kg, respectively) and transcardially perfused with 4% paraformaldehyde and 2% glutaraldehyde (300 cc) after infusion of 0.9% NaCl (300 cc). Upon the completion of the perfusion, the temporal bones and the kidneys were meticulously dissected out. Otic capsules and the kidneys were soaked in 4% paraformaldehyde and 2% glutaraldehyde solution in separate vials. The tip of the cochlea was clipped and the stapes were removed from the otic capsules. From these sites, the vestibular end organs were gently perfused with 2% glutaraldehyde and 4% paraformaldehyde with a micropipette under the dissecting microscope at least three times during the 6 hours they were embedded in the solution. Following dissection of the end organs, these tissues were washed out three times (each ~15 minutes) with 0.1% phosphate buffer before and after they were treated with 0.1% OsO_4 for 45 minutes. Three consecutive treatments of alcohol dehydration, each lasting 10 minutes, were performed. For subsequent analysis, the kidneys were embedded in paraffin blocks and the end organs in plastic (araldite).

The cristae were cut vertically into 1μm thick sections starting from the planum semilunatum (PS) to the center (C) (300-500 μm) with the aid of an ultramicrotome (MT2-B Dupont). To ensure a well-distributed sample from each of the cristae, the first 5 sections of each 25 were mounted onto individual glass slides; the next 20 sections were discarded. This was repeated throughout the entire first half of each crista (approximately 500 μms) as one half supplied more than an adequate amount of sections for analysis. The PCA were stained with 0.1 % Toluidine Blue.

Paraffin embedded kidneys were cut into 25 μm sections stained with hematoxilene-eosin. Both the PCAs and kidneys were evaluated under light microscopy.

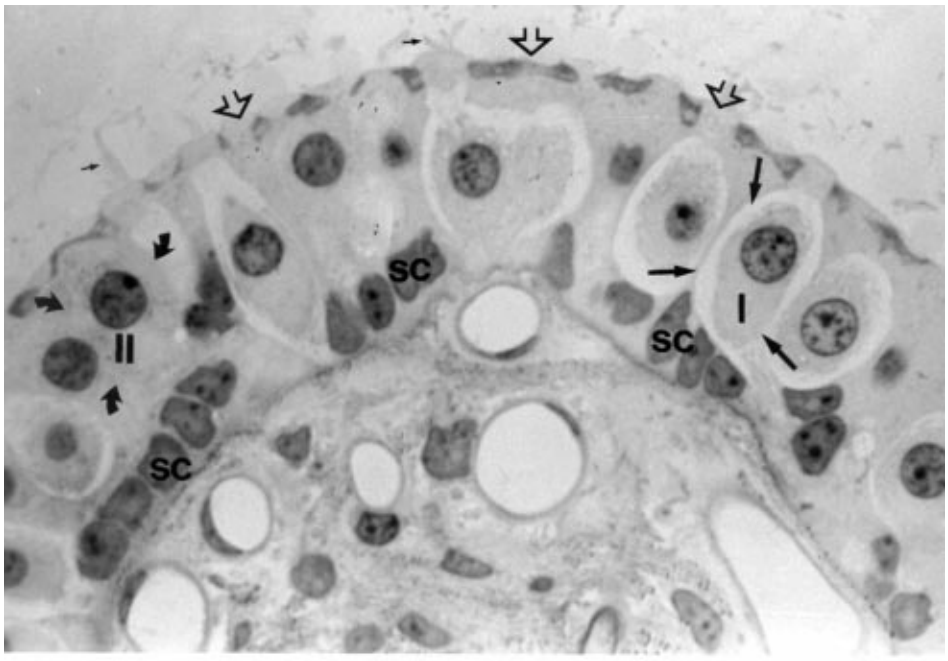

Figure 1. Potomicrograph of the PCA from a control animal showing the normal appearance of the sensory NE.
A circular shaped Type I (I) HC surrounded with a nerve calyx (straight arrow) and a type II (II) HC cylindrical in shape (curved arrow). The basal lining of supporting cells (sc) exhibits a monolayer between this HC layer and stroma. Cuticular plates of the HCs facing the endolymphatic space are continuous (open arrows). Bundles of cilia can be observed across the surface of the crista (small arrows).

Results

Morphology of the Chinchilla Vestibular Epithelium

In mammals, the vestibular NE basically consists of three types of cells. Type I and Type II HCs and the supporting cells (Figure 1). The HCs are situated in the upper two thirds of the NE. The HCs can be differentiated into two groups in terms of their morphology and innervation. Type I HCs are circular in shape and they are surrounded with a herve calyx. More than one Type I HC enveloped by one nerve calyx is common at the summits of the crista in the more central regions (10, 11). Type II HCs are rectangular in shape and they receive button nerve endings. Stereocilia are the hairs of the cells that face the endolymphatic lumen. The supporting cells are basically cuboidal in shape with an ovoid nucleus; the supporting cells exist as a monolayer in the lower one third of the NE.[10,11]

Gentamicin Ototoxicity

The three different doses of GM used all rendered a similar, non-specific morphology within the NE when examined on the first day post-treatment. The NE

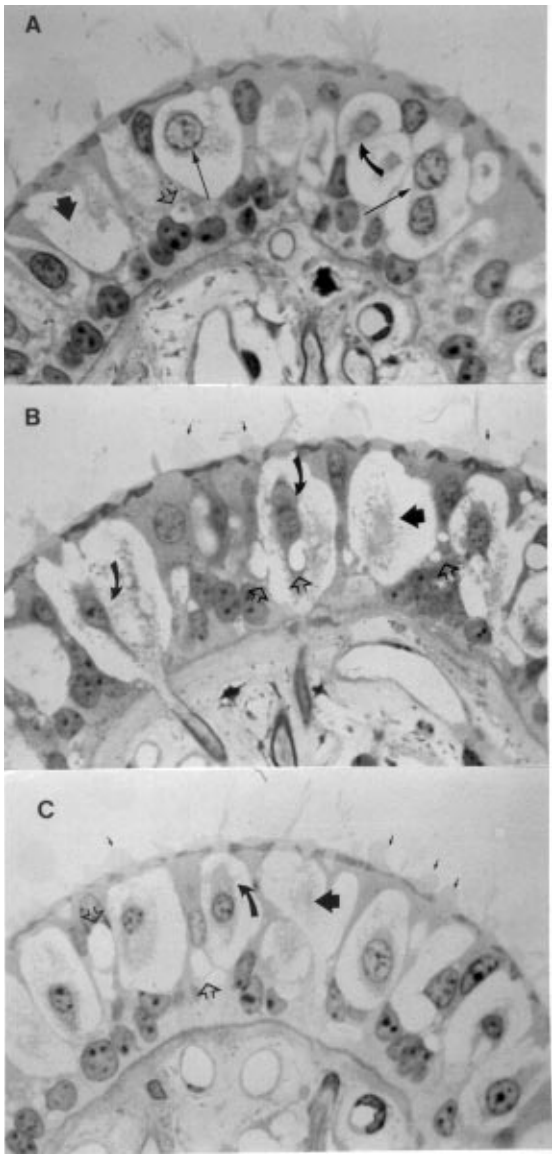

Figure 2. Representative sections of the PCA of animals that were treated with 120, (A), 60 (B) and 30 mg/kg (C) GM. The NE shows similar morphology with the three different doses. There are early signs of cell death such as nuclear swelling (long arrows), HC cytoplasmic vacuolazation (open arrows) and shrinkage (curved arrows). Cytoplasmic protrusions (small arrows) and empty calyces (solid arrow) can be seen.

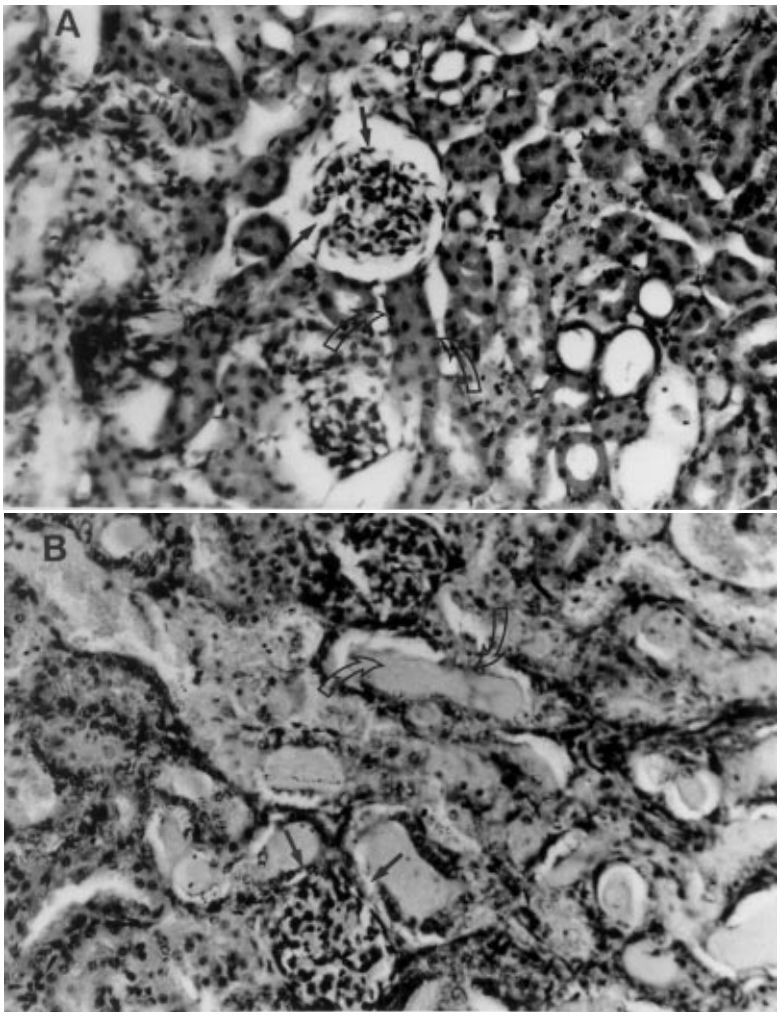

Figure 3. Representative photomicrapraphs of kidney from a control chinchilla (A) and chinchilla treated with 120 mg/kg GM for six days (B). Glomeruli (solid arrow), normal proximal tubules (A, curved arrow) and proximal tubular necrosis (B, curved arrows) are seen.

displayed HCs with vacuoles in the cytoplasm, apical surface extrusions toward the endolymphatic space and a few empty nerve calyces (Figure 2).

Gentamicin Nephrotoxicity

At the higher doses of GM, the kidney histopathology revealed prominent proximal tubular damage (Figure 3) whereas the 30 mg/kg dose appeared to induce less damage to the proximal tubular epithelia. These morphological observations correlated well with the monitored blood levels of urea nitrogen and creatinine. The results from the quantitative blood chemistry are shown in Figure 4. Both BUN and creatinine levels were found to be normal in blood samples from control animals. Although the animals receiving the 30 mg/kg dose of GM showed an initial, modest elevation in both BUN and creatinine levels, these levels returned to control values when monitored during the post-treatment period. Some of the animals expired shortly after (within 3-5 minutes) the GM injection on the third or fourth day of treatment. These animals presented signs of acute respiratory failure such as dyspnea or apnea.

Discussion

GM Ototoxicity

In the current study, all three doses of applied GM (120, 60 and 30 mg/kg) yielded nonspecific changes in the PCA NE on the first day after the complete treatment regimen. The minimum dose to establish a clear ototoxic effect is known to show intervariability between species (12). Neurophysiological recordings in the cat determined that a dose of 40 mg/kg of GM must be administered daily for at least ten days to induce pathological ototoxicity (13). Daily systemic administration of GM in a dose range of 100-120 mg/kg for 10 days established vestibulotoxicity in the guinea pig (14), whereas 30-60

mg/kg/day for ten days is sufficient to produce ototoxic effects in the squirrel monkey (15). In the chinchilla however, our two highest doses, 60 and 120 mg/kg/day, resulted in overwhelming mortality rates as early as third or fourth day of treatment. However, administration of 30 mg/kg/day of GM induced similar pathology on the first day PT. Therefore we believed that 30 mg/kg/day of GM was an ototoxically efficient and ultimately safer dose for further studies in this mammalian model (Figure 4).

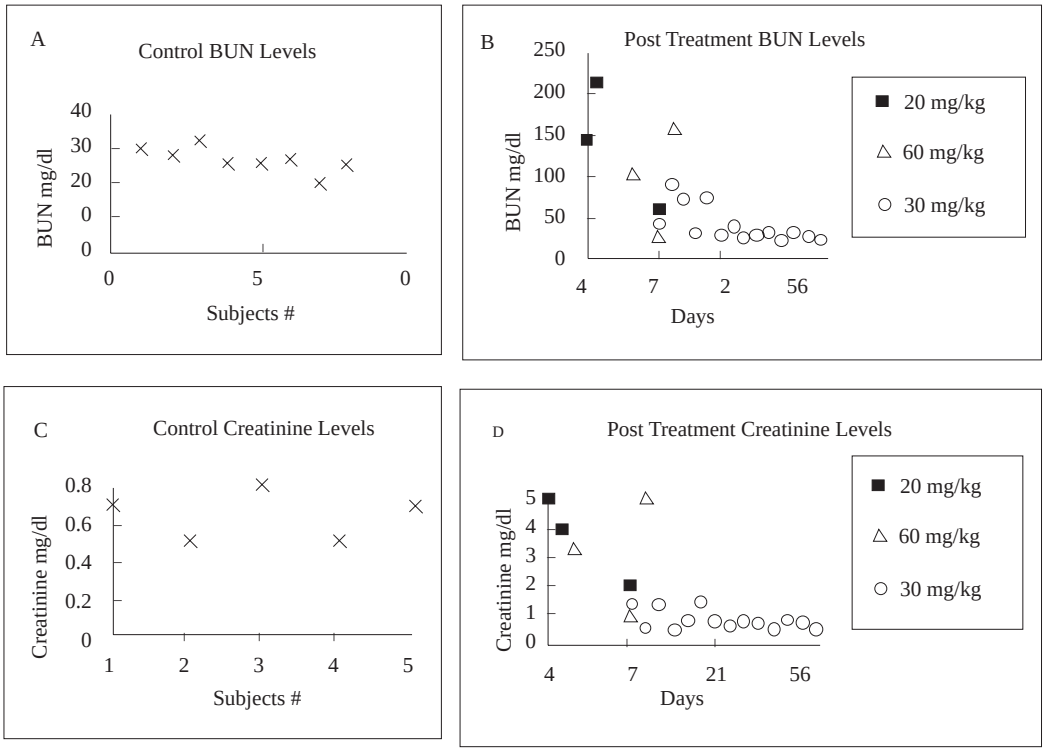

Figure 4. The results from the quantitative blood chemistry are shown in chart form. A and C show the control values for BUN and Creatinine. B and D represent post–treatment values respectively in the chinchilla.

GM Nephrotoxicity

The reversible nephrotoxic side effect of this aminoglycoside antibiotic is observed earlier than its ototoxic effect[7]. The comatose symptoms observed in our animals at the higher doses of GM most probably reflect toxic levels of retained urea due to acute renal failure and/or respiratory due to neuromuscular blockade.[12] Rybak observed overwhelming systemic toxicity which was incompatible with the survival of the animals when he was investigating developmental ototoxicity in the neonatal rats.[16] GM's dose-response relationship was consistent with early deterioration of kidney function at the higher doses in the current study. Evidence of malfunctioning kidneys was confirmed with observed morphological proximal tubular damage (Figure 3) and increase in BUN and creatinine levels (Figure 4). Respiratory failure due to neuromuscular blockade and systemic toxicity are associated complications of aminoglycoside-induced ototoxicity (4, 7, 8, 16, 17). While we could not specifically determine whether nephrotoxicity or GM-induced neuromuscular blockade inidvidually caused the loss of animals at the higher doses of GM, it is likely that both of these life-threatening conditions were present and cooperating leading to the observed symptoms.

In conclusion, systemic GM administration of 30, 60 and 120 mg/kg/day can induce similar morphological changes in the crista NE of the chinchilla in the early stages of toxicity. However, administrations of higher doses display acute renal failure and/or neuromuscular blockade resulting in inevitable loss of animals. Therefore, we recommed 30 mg/kg/day dose as an efficient tool to investigate ototoxicity in the chinchilla.

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