Light Microscopic Evidence of Hair Cell Regeneration After Gentamicin Toxicity in Chick Cochlea

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- This study examines the temporal pattern of hair cell loss in the chick basilar papilla following ten days of gentamicin administration in hatching chicks. Chicks were subsequently killed at ages 11, 18, 25, and 32 days. The basilar papillae were embedded in plastic and serially sectioned for light microscopic analysis. Hair cell counts were obtained at 100-μm intervals throughout the length of the papilla. Significant hair cell loss was documented basally in the 11-day-old chicks, and spread apically over time to maximal loss in the 18-day-old animals. Relative to the control chicks, there was a 36% hair cell loss in these animals. Interestingly, there appears to be a progressive partial recovery of the normal hair cell counts in the 25- and 32-day-old animals.

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It has been well established that therapy with aminoglycosides can cause cochlear damage. Several stud-
ies have noted that, initially, the most significant damage occurs at the level of the hair cells. In particular, aminoglycosides appear to damage hair cells in the basal cochlea, with further extension apically over time or with continued drug administration. Very few studies have attempted to define more completely the progression of an aminoglycoside-induced cochlear injury. Certainly, further studies might provide insight into such clinical phenomena as unilateral loss of hearing, delayed onset of hearing loss, and the reversibility of hearing loss. The purpose of this study was to examine histologically the temporal patterns of hair cell loss in the chick basilar papilla following gentamicin administration. Particular attention was directed toward defining the rapidity of hair cell loss, when hair cell loss peaked, and if recovery of hair cell numbers occurred.

SUBJECTS AND METHODS

Subjects

Thirty-two domestic chicks were used and 40 cochleas were examined histologically. The experimental group consisted of 16 hatching chicks, which were given single daily subcutaneous injections of gentamicin at a dose of 50 mg/kg/d for ten days. This dose was chosen on the basis of previous data indicating substantial and graded hair cell loss with a 25-day course of 50 or

Fig 1.—Experimental design.

![Experimental design diagram]

32 Newborn Chicks

Experimental

(n = 16)

Gentamicin, 50 mg/kg/d for 10 d

Killed at ages 11, 18, 25, and 32 d

Control

(n = 16)

Killed at ages 11, 18, 25, and 32 d

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100 mg/kg of gentamicin, tobramycin, or amikacin. These chicks were subcutaneously killed and their cochleas studied histologically at one day (aged 11 days), one week (aged 18 days), two weeks (aged 25 days), and three weeks (aged 32 days) postinjection. Similarly, 16 hatchling chicks were raised as controls. These were killed and studied at identical ages of 11, 18, 25, and 32 days. A summary of these various groups is shown in Fig 1.

Fixation and Tissue Preparation

Tissue processing and analysis techniques were similar to those used previously by this laboratory. Immediately after death, induced by intraperitoneal pentobarbital (Nembutal) overdose, the chicks were decapitated. A direct bilateral intralabyrinthine perfusion of 1% paraformaldehyde and 2.75% glutaraldehyde fixative in 0.12 M phosphate buffer (pH 7.2) was performed. After two to five days of immersion in this fixative, the basal papillae were dissected from the temporal bones. The papillae were washed in a phosphate buffer and postfixed in 2% osmium tetroxide. Following osmication, the papillae were dehydrated in a graded methanol series prior to embedding in epoxy resin. Epoxy resin blocks were then sectioned transversely to the longitudinal axis of the papillae in the proximal to distal (basal to apical) direction. A group of three or four serial sections, each 4 μm in thickness, were obtained at 100-μm intervals along the length of the papilla. These sections were subsequently mounted and stained with toluidine blue.

Quantitative Hair Cell Analysis

Quantitative hair cell analysis at each level of the basilar papilla was performed by viewing each section under an oil immersion objective (×40) at a total magnification of ×500. In order to maintain consistency and reliability between hair cell counts, counting criteria similar to those used previously were established: the presence of a well-formed cell body, extension of the cell to the cuticular plate, and identifiable stereocilia. Three 4-μm sections were analyzed at each 100-μm interval and the average number of hair cells per section was recorded. Counts were expressed as a function of the distance from the base, and then normalized across animals by converting to percent of the total length of the papilla in 5% increments.

RESULTS

Normal Anatomy of the Chick Basilar Papilla

The anatomy of the chick basilar papilla (cochlea) has been described in detail previously. Figure 2 is a low-power photomicrograph demonstrating the general appearance of the chick’s cochlea seen in a 4-μm-thick transverse section from the midportion (50% of the distance from the proximal or basal tip). The tegument vasculosum is demonstrated superiorly, while the tectorial membrane with underlying basilar papilla can be seen inferiorly. The darkly stained hair cells can be seen along the free margin of the basilar membrane. Figure 3 is a higher-power photomicrograph of the basilar papilla taken from the same region. As described by Retzius, two types of hair cells are found in the papilla: tall hair cells located medially and short hair cells located laterally toward the free edge of the basilar membrane. These “tall” and “short” avian hair cells may homologize with mammalian “inner” and “outer” types. For purposes of this study, both cell types were combined for total hair cell counts. In contrast to mammalian preparations, the avian cochlea generally shows increasing numbers of hair cells across its width as one moves toward the apex. Cell counts revealed a small number (five to seven) of hair cells across the basilar membrane at the base, gradually increasing in number as the apex was approached, until a maximum of 25 to 30 cells were seen at approximately 90% of the length. Subsequent graphic representations of these hair cell counts will have the total mean hair cell counts of the cochleas as the ordinate and the length of the cochlea expressed in percentages from base to apex as the abscissa.

Normative Hair Cell Counts

Figure 4 demonstrates the various control data groups. Each curve represents the combined mean hair cell counts of three cochleas at a particular age group—11, 18, 25, or 32 days. This graph reveals the remarkable consistency of the control cochleas’ mean hair cell counts, and indeed, when these control cochleas are combined into a single curve, the standard error of the mean at any measured
point along the curve is never greater than plus or minus one hair cell. For this reason, our control data will hereafter be combined into a single cumulative curve of 12 cochleas taken to represent the normative hair cell counts.

**Experimental Hair Cell Counts**

In the 11-day-old animals, significant hair cell loss, particularly in the basal 50% of the cochlea, was noted. Additional hair cell loss occurred in the 18-day-old animals as the damage spread apically to involve greater than 60% of the length of the cochlea. These data are summarized in Figs 5 and 6. Figure 7 shows a photomicrograph of representative tissue taken from the midportion (50% region) of an 18-day-old experimental animal. When compared with Fig 3, the loss of hair cells is appreciable, especially in the more lateral position of the “shorter” hair cells.

In the 25-day-old animals, hair cell loss was present throughout the length of the cochlea (Fig 8), but there was a trend toward increasing mean hair cell counts in the midportion of the cochlea relative to the 18-day-old animals. This trend is even more apparent in the 32-day-old animals (Fig 9). A representative tissue sample from the midportion of a 32-day-old cochlea (Fig 10) clearly demonstrates the recovery toward a normal complement of hair cells compared with an 18-day-old cochlea (Fig 7).

Thus, our experimental data suggest a period of maximal hair cell loss at 18 days of age, with a partial restoration of hair cell counts in the midcochlea of the 25- and 32-day-old chicks. For example, the mean hair cell count at the 45% region of the 18-day-old cochleas was seven (range, five to ten), whereas this value was 14 (range, ten to 19) for the 32-day-old animals. At the 50% region a similar difference was noted: a mean hair cell count of four (range, five to 12) vs 16 (range, eight to 20). This finding is further demonstrated in Fig 11, where total hair cell counts between the 45% and 55% cochlear regions have been plotted for the 18-day-old and 32-day-old animals. In this histogram, the total hair cell counts have been expressed as a percentage of the normative hair cell counts for that region of the cochlea. In the 18-day-old group this figure is 36%, while in the 32-day-old group it is 73%, representing a twofold increase in the average hair cell counts.

One possible explanation for this apparent restoration in hair cell numbers is that the change is due to our criteria for including a hair cell in the
counts. That is to say, hair cells that had undergone sublethal damage and did not meet the counting criteria at 18 days were sufficiently repaired by 32 days to be included in the hair cell counts. To further evaluate this hypothesis, hair cell counts were repeated using very liberal criteria in the 18- and 32-day-old animals as well as in the control animals. Any portion of a cell body (without regard to presence of a cuticular plate or stereocilia) along the surface of the basilar papilla was counted; thus, even severely damaged cells were included. As might be expected, the mean hair cell counts rose in all three groups. Importantly, however, there still remained a significant difference in the number of hair cells among the 18-day-old, 32-day-old, and control cochleas. For example, the mean hair cell count at the 45% region of the 18-day-old cochleas was 11 (range, nine to 16), whereas this value was 22 (range, 15 to 25) for the 32-day-old animals. At the 50% region a similar difference was noted: a mean hair cell count of 14 (range, nine to 22) vs 22 (range, 11 to 28). Thus, when virtually all the cells in the basilar papilla of the 18- and 32-day-old animals were included in the counts, there was still an increase in the number of hair cells in the older animals.

**COMMENT**

The chicken preparation used in this study has proven to be a useful model for ototoxicity studies. Developmentally, the chick inner ear (unlike that of many rodents) resembles the human ear in that it is nearly fully developed at birth. In addition, there is a wealth of anatomic, physiologic, and behavior data on the organization and development of the chick inner ear and central auditory pathways. Previous studies have demonstrated cytopathic changes similar to changes in mammals with aminoglycoside administration. Pragmatically, the chick’s short gestational period (20 to 21 days) and inexpensiveness as a laboratory animal make it an ideal subject for experimentation.

As previously mentioned, numerous studies have described the progressive nature of hair cell loss in various animals with aminoglycoside intoxication. Others have also noted the continuation of hearing loss and hair cell degeneration after the cessation of antibiotic administration as observed in this study. Although controversial, it has been postulated that the continuation of hearing loss or its delayed onset may be related to an accumulation of aminoglycoside in the perilymph, due to a prolonged half-life there relative to the serum half-life of aminoglycoside.

The restoration of hair cell counts in this study was unexpected. As previously described, this observation does not appear to be an artifact of
the counting criteria. One theoretical mechanism could involve migration of hair cells into the damaged portions of the basilar papilla. This mechanism does not appear tenable, however, as there is a significant difference in the total number of hair cells in the basilar papilla of the 18- and 32-day-old animals. A shift in the positions of the hair cells along the basilar papilla would not increase the number of hair cells involved and, therefore, would not explain the observed difference in total hair cell counts. Another theoretical mechanism could involve the differentiation of supporting cells into more specialized hair cells to replace irreparably damaged ones. Finally, an intriguing, but generally discarded, mechanism could involve the actual regeneration of hair cells from a dormant precursor population.

Hair cell regeneration would involve the actual mitotic production of new hair cells. Corwin\textsuperscript{14}\textsuperscript{15}\textsuperscript{17} has described evidence of postembryonic production of hair cells in a number of anamniotes such as rays, sharks, and toads. However, to date, such postembryonic hair cell production has not been described in an anamniote species. Bredberg\textquoteright;s\textsuperscript{18} study of human noses that hair cell populations of the ear did not significantly increase beyond the size observed in newborns. This lack of continuous postembryonic production of hair cells does not preclude, however, the possibility that regeneration could be stimulated in the damaged cochlea. It should be noted that in reviewing our histologic material, little evidence of hair cell mitotic activity was seen. Further studies are now in progress to investigate this hypothesis.

In addition to possible hair cell regeneration, reversal of ototoxicity might result from recovery from an abnormal physiologic state. For example, Takada and Schacht\textsuperscript{19} found that the toxicity of gentamicin as reflected by serial measurements of the cochlea microphonic in guinea pigs could be initially antagonized competitively with perfusion of calcium into the perilymph. He suggested a biphasic mechanism of aminoglycoside ototoxicity; an initial, reversible action competitive with calcium ions, and a second step, irreversible and noncompetitive. This second mechanism may involve the specific binding of the drug with the cell membrane phospholipid phosphatidylinositol.

Mechanisms discussed above may be important to the clinical observations regarding the not-infrequent recovery of hearing loss from aminoglycoside toxicity. Fee\textsuperscript{14} notes that 55% of 138 patients demonstrated recovery from cochlear toxicity over a time span of one week to six months. Moffat and Ramsden\textsuperscript{20} provide a case report of a patient demonstrating marked improvement in hearing eight months after completing a course of tobramycin and gentamicin therapy. Those patients showing a rapid reversal of ototoxicity may be recovering in a manner compatible with the hypothesis of Takada and Schacht,\textsuperscript{19} those recoveries occurring later, however, are more difficult to explain. Further studies using titrated thymidine after drug ototoxicity or other cochlear insult in the chick may produce further evidence for the possibility of postembryonic hair cell regeneration.

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