STATE-OF-THE-ART

Ototoxicity in preterm infants: effects of genetics, aminoglycosides, and loud environmental noise

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Majority of hearing-loss cases with extremely preterm infants have no known etiology. There is a growing concern that the administration of aminoglycoside treatment in the noisy environment of the Neonatal Intensive Care Unit (NICU) may lead to hair-cell damage and subsequent auditory impairments. In addition, several mitochondrial DNA mutations are known to have been associated with aminoglycoside-induced hearing loss. This review provides a systematic analysis of the research in this area and elucidates the multifactorial mechanisms behind how mitochondrial DNA mutations, aminoglycosides and loud noise can potentiate ototoxicity in extremely preterm neonates. Recommended steps to minimize the risk of ototoxicity and improve clinical care for NICU infants are discussed.

Journal of Perinatology advance online publication, 9 August 2012; doi:10.1038/jp.2012.105

Keywords: aminoglycosides; mitochondrial DNA mutations; auditory; noise; ototoxicity; preterm infants

NEONATAL HEARING

Early auditory development

Development of the auditory system begins as early as 3–6 weeks of gestation age (GA).¹,² By ~25 weeks GA, the structural aspects necessary for audition are intact, and the fetus can already perceive and respond to low-frequency sounds passing through amniotic fluid.³ The neurosensory pathways of the auditory system are known to develop later in gestation, eliciting brainstem and cortical auditory evoked responses at around 28 weeks GA.⁴,⁵

Many of the sounds that are audible in the womb are generated internally by the mother’s respiration, digestion, heart rhythms and physical movements.⁶,⁷ Fetuses, however, can also respond to sounds outside of the womb. Animal and human studies have used ultrasound technology to observe fetuses’ behavioral responses to sound stimuli.⁸,⁹ For example, Hepper and Shahidullah⁸ used ultrasound technology to observe fetuses’ behavioral responses to low-frequency sounds outside of the womb. Animal and human studies have shown that loud noise can lead to unwarranted transient changes in the physiologic, motor and state-related systems of extremely preterm neonates.¹⁶ This vulnerable population of newborns is especially sensitive to noise, because their ability to self-regulate and filter noxious stimuli is extremely limited. It has therefore been suggested that excessive exposure to loud noise during the neonatal period can heighten the risk for sensory deficits and developmental disabilities (for a review, see ref. 17).

The transition from the womb to the NICU environment

The well-structured course of auditory development is severely interrupted when a preterm infant enters the noisy world of the Neonatal Intensive Care Unit (NICU). First, the hearing experience in the NICU, where sounds are transmitted through air, is very different from the transmission of sounds through the amniotic fluid in the womb. In addition, the type of sounds and levels of noise typically present in the NICU are very different from those present in utero, putting preterm infants at risk for exposure to sound frequencies that they are not yet ready to process. Noises in the NICU come from fans, ventilators, telephones, pagers, doors, loud conversations and intermittent alarms. Previous studies have shown that the median noise levels in the NICU range from 55 to 67 dBA with intermittent peaks ranging from 75 to 120 dBA,¹¹-¹⁴ which exceeds the recommended noise level from the American Academy of Pediatrics (45–55 dBA).¹⁵ Studies have shown that loud noise can lead to unwarranted transient changes in the physiologic, motor and state-related systems of extremely preterm neonates.¹⁶ This vulnerable population of newborns is especially sensitive to noise, because their ability to self-regulate and filter noxious stimuli is extremely limited. It has therefore been suggested that excessive exposure to loud noise during the neonatal period can heighten the risk for sensory deficits and developmental disabilities (for a review, see ref. 17).

Hearing loss in preterm neonates

Hearing loss is one of the most common health problems affecting one in 700–1000 newborns.¹⁸ The rate of hearing loss in preterm infants is between 2% and 15%, with the majority of the cases having no known etiology.¹⁹,²⁰ Classifications for hearing loss include: genetic or non-genetic, pre-lingual or post-lingual, and syndromic or non-syndromic. Overall, 50% of congenital/pre-lingual sensorineural hearing impairments are attributed to genetic factors, and 20–25% of cases are due to identifiable environmental causes.
such as perinatal and/or postnatal infections due to viruses, acoustic or cerebral trauma affecting the cochlea, or ototoxic drugs such as aminoglycosides. Additionally, non-syndromic hearing impairment (NSHI) accounts for ~80% of cases of heredity deafness. However, a majority of hearing-loss cases remain unknown.

**AMINOGLYCOSIDES AND LOUD NOISE**

Preterm infants have underdeveloped immune systems that are inefficient at preventing infection. A common infection seen in NICU infants is sepsis, a condition where the bloodstream is overwhelmed by bacteria. Depending on whether the sepsis onset is early or late, often a combination of aminoglycosides, β-lactam and other various pharmaceuticals are used for treatment. Aminoglycosides are a class of antibiotics utilized against certain types of bacteria, specifically Gram-negative infections. The most common aminoglycoside used in the NICU is gentamicin, and it has often been the aminoglycoside of choice because of its low cost and effectiveness against most aerobic Gram-negative bacilli.

Although aminoglycosides are vital for reducing bacterial infections, they are also known to have adverse side-effects. In general, aminoglycosides are toxic to the eighth cranial nerve (auditory nerve) and the kidneys. Studies have shown that aminoglycosides progressively accumulate in the endolymph and perilymph of the inner ear, which may result in temporary and/or permanent hearing loss.

There are points throughout development where aminoglycosides may be more ototoxic than others. For example, Bernard found that exposure to aminoglycosides during the neonatal period can alter auditory responses in the kitten model. A striking finding in this study was that the immature ear was more susceptible to cochlear damage than the adult’s ear, revealing a sensitive period for aminoglycoside-induced toxicity.

Interestingly, this sensitive period coincides with the final stages of anatomical development and differentiation within the cochlea.

There is a growing concern that the administration of aminoglycoside treatment in a noisy NICU environment can result in adverse auditory outcomes. This combination may be particularly harmful considering its occurrence during a critical period for auditory brain development. Thus, the potentiating effect of noise and aminoglycosides could possibly account for some of the unknown etiology reported with hearing-loss cases among this population. The majority of research in this area, however, is derived from animal studies.

Darrouzet and Limasobrinhoe found that animals who received aminoglycosides appeared to be more susceptible to noise-induced hearing loss. This study was soon followed by Gannon and Tso et al. who described the potentiation of aminoglycoside-induced toxicity by simultaneous exposure to noise. These early discoveries have laid the groundwork for many follow-up studies examining the combination of noise and aminoglycosides under various conditions (see Figure 1).

The commonly cited animal studies in this field are presented in Figure 1. With the exception of one study by Fernandez et al., all sixteen studies shown in Figure 1 found a potentiating effect between noise and aminoglycosides, with the majority of studies reporting hair-cell damage.

Although the combination of noise and aminoglycosides has been well studied, there are still several inconsistencies across study designs that limit our ability to draw definite conclusions. Much of the variability across studies is because of the various types of aminoglycosides, dosage amounts, level and duration of noise exposure, animal model utilized and age at testing.

![Figure 1. Shown are animal studies (shapes) examining the combination of aminoglycosides and noise superimposed on human studies (yellow shade) reporting the range of noise peaks in the Neonatal Intensive Care Unit (NICU) in reference to the recommendations for noise levels (red dashed lines) set by the American Academy of Pediatrics (AAP). Our analysis reveals a complete overlap between the noise levels in the animal studies and the noise levels experienced by NICU infants; therefore, the adverse auditory outcomes evident in the animal model are highly generalizable to humans.](https://example.com/figure1.png)
controlled and age-restricted studies need to be completed in an effort to make the results more generalizable to the newborn population in the NICU.

NOISE POTENTIATES AMINOGLYCOSIDE TOXICITY: A POSSIBLE MECHANISM

The complete mechanism by which noise potentiates aminoglycoside ototoxicity is still unfolding. However, for the purpose of this paper, we propose a possible mechanism for how noise and aminoglycosides combine together to potentiate ototoxicity. The proposed mechanism is supported by the literature and is illustrated in Figure 2. When the animal/infant is exposed to excessive noise, the basilar membrane in the cochlea vibrates more vigorously resulting in an immense amount of hair-cell movement that can cause hair-cell and structural damage within the cochlea. As noise increases, it creates more vibration on the basilar membrane—this increases both the number of hair cells that are stimulated and the rate at which they generate nerve impulses.

Loud noises cause more hair cells to be stimulated, thereby allowing for more mechanoelectrical transduction (MET) to occur at the apical surface of the hair cells. Normally, when MET channels open, an array of ion-channel kinetics take place resulting in depolarization. However, once aminoglycosides have entered the endolymph of the scala media, they can permeate through the MET channels, thereby blocking the rapid depolarizing transduction current. Figure 2 illustrates how exposure to loud noise increases the open probability and current through MET channels which, in turn, results in a greater aminoglycosides uptake within the hair cells. Other mechanisms that potentiate ototoxicity at cellular levels are likely.

COCHELAR SUSCEPTIBILITY

Often, damage to hair cells progresses from the base of the cochlea to the apex. In vitro and in vivo studies have shown the basal outer hair cells to be more susceptible to damage from gentamicin. It appears that aminoglycosides preferentially target the base of the cochlea, an area that in early postnatal stages has the highest open probability of the MET channels. In utero, the fetus hears predominately low-frequency sounds that stimulate the apex of the cochlea; however, while in the NICU, the infant is unwillingly exposed to high-frequency sounds coming from phones and alarms that stimulate one of the most vulnerable areas of the cochlea – the base. This unwarranted stimulation in an extremely sensitive area at the base of the cochlea, where the open probability of MET channels is most heightened, is especially dangerous because it allows for significantly more aminoglycoside uptake into the hair cells of this region. Taken together, it is likely that the administration of aminoglycosides in a loud NICU environment during a sensitive period of auditory development is an undesirable recipe for hearing loss.

AMINOGLYCOSIDES AND GENETICS

Mitochondrial DNA mutations

Hearing loss can occur from mutation(s) in a single gene or from a combination of mutations in various genes. Mitochondrial DNA (mtDNA) contains 37 genes, all essential for proper mitochondrial function. However, the mitochondrial 12S rRNA is a prime target for mutations associated with aminoglycoside-induced NSHI. The identified NSHI mutations in the 12S rRNA gene include the following: A1555G, T1095C, C1494T and 961 mutations. Previous studies have shown a genetic link between aminoglycoside-induced ototoxicity and mutations in the human 12S rRNA gene. Therefore, with the above mitochondrial mutations, it is believed that aminoglycoside is the predominating modifying factor for hearing loss.
Although the exact mechanism of ototoxicity and mtDNA mutations is not fully understood, several studies have shown that human mitochondrial 12S rRNA alter the binding properties of aminoglycosides. Variants within the mitochondrial mutations make the mitochondrial ribosome more similar to bacterial ribosomal RNA, resulting in the cells being more susceptible to aminoglycoside-induced damage. As discussed earlier, aminoglycosides are highly concentrated in the perilymph and endolymph of the inner ear. Because the cells in the inner ear are rich with mitochondria (owing to their high metabolic activity and role in sensory transduction), they may be more predisposed to aminoglycoside-induced damage. Consequently, exposure to aminoglycosides can potentiate hearing loss in patients with mitochondrial 12S rRNA mutations. In his hypothesis, mitochondrial translation defects result in a reduction in ATP production and an increase in reactive oxygen species; cell death occurs and eventually leads to hearing loss and/or deafness. Although this theory has not been formally tested, it is likely that the addition of NICU noise can further potentiate ototoxicity in subjects with mtDNA mutations exposed to aminoglycosides during the neonatal period. More research in this area needs to be completed in an effort to prevent drug-induced hearing loss among the preterm infant population.

Mitochondrial DNA mutations and NICU infants

The exact prevalence of mtDNA mutations among infants born prematurely is unknown. Ealy et al. examined the prevalence of mitochondrial mutations in a population of 703 former NICU graduates from Iowa Children’s Hospital and found the frequency of these variants was ~1.8%. In addition, they did not find hearing loss in patients at risk. Although this study showed a relatively small prevalence with no hearing loss associated, more studies need to be completed that examine the prevalence of mtDNA mutations in preterm infants receiving aminoglycosides. In addition, premature infants are exposed to NICU noise, which may further increase the ototoxicity experienced by individuals with mtDNA mutations while taking aminoglycosides.

It is likely that when preterm infants who carry the mitochondrial mutation are exposed to not only aminoglycosides but also to loud NICU noise, the following occurs: (1) more hair cells are recruited because of the loud noise level; (2) more aminoglycosides enter the hair cells and alter the binding properties; (3) mitochondrial translation defects occur resulting in a reduction in ATP production and an increase in reactive oxygen species; (4) cell death occurs and eventually leads to hearing loss and/or deafness. Although this theory has not been formally tested, it is likely that the addition of NICU noise can further potentiate ototoxicity in subjects with mtDNA mutations exposed to aminoglycosides during the neonatal period. More research in this area needs to be completed in an effort to prevent drug-induced hearing loss among the preterm infant population.

IMPLICATIONS FOR CLINICAL CARE

Preventing the combination between noise levels and aminoglycosides in the NICU is an arduous task. However, there are several steps that can be taken to improve clinical care and reduce ototoxicity, including reducing NICU noise, performing genetic testing, attaining family history of mtDNA mutations and increasing the safety of aminoglycosides through better pharmacological innovations.

NICU noise

An immense amount of the loud noise present in the NICU can be easily reduced, or eliminated, with some design modifications to the NICU environment. For example, implementing (1) a silent alarm system; (2) a noise-level meter at the bedside; and (3) a private bed suite (for reviews on ways to improve the NICU environment).
environment, see ref. 67, 68). These rather small adaptations to the hospital environment can make a huge impact on patient care and can increase the awareness among doctors, nurses, parents and caregivers about the acoustic environment surrounding infants taking aminoglycosides.

Genetic testing
NSHI is caused by mutations in mtDNA and is transmitted by maternal inheritance. It is therefore advisable that pregnant women at-risk for preterm birth should be given a genetic test for 12S rRNA mutations. In the very least, a thorough family history inquiring about mtDNA mutations should be completed. This knowledge will help to guide the physician toward choosing an alternative drug rather than aminoglycosides, or to closely monitor the dosage-level owing to the increased risk of hearing impairment. Those who are positive for 12S rRNA mutations should be warned about the risk for aminoglycoside-induced ototoxicity and told to avoid the use of these drugs if possible.

Aminoglycoside dosage and safety
It is also important to optimize the dosage of aminoglycoside given to NICU infants (for review on this topic see69,70). There are likely many pharmacological improvements that should be made to reduce aminoglycoside uptake in the hair cells. Alharazneh et al.52 suggest that limiting permeation of aminoglycosides through MET channels or preventing their entry into endolymph are potential therapeutic targets for preventing hair-cell death and hearing loss. More pharmacological research needs to be done in this area in an effort to reduce the potentiating effect of aminoglycosides on NICU noise and mtDNA mutations.

CONCLUSIONS
This review proposes an integrative model by which loud noise, mtDNA mutations and aminoglycosides result in repeated toxic reactions to structures of the inner ear, accounting for some of the NSHI reported in infants born prematurely. Although it is often difficult to determine the exact reason for hearing loss among this population, there is growing evidence to suggest that the unique combination of environmental, genetic and pharmacological factors during NICU hospitalization can be very detrimental to developing auditory system. Steps need to be taken to minimize the impact of this adverse combination.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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