ABSTRACT

Reversibility of Aminoglycoside Induced Ototoxicity

Akansha Mohan

Director: Dr. Rizalia Klausmeter, Ph.D

Mentors: Dr. Barry Strasnick, M.D.; Dr. Sal Taliercio, M.D.; Dr. Seth McAfee, M.D (Eastern Virginia Medical School)

Although gentamicin is a useful antibiotic, it results in permanent auditory and vestibular ototoxicity by damaging inner ear hair cells. *A.aurita* jellyfish ephyrae have functionally and morphologically similar hair cells to humans. They show permanent hair cell loss after exposure to 3.5 mM dose of gentamicin.

Research objectives include determining whether lower doses of gentamicin (1mM) administered over a longer period of time can have therapeutic benefit free of permanent toxicity, while also evaluating the suitability of *A.aurita* jellyfish ephyrae to model aminoglycoside induced ototoxicity.

Methods: 30 controls were exposed to artificial sea water (ASW) for one hour, and subsequent groups of 30 ephyrae were exposed to 1 mM gentamicin solutions for 1h, 24h, or 48h. Ephyrae were allowed to recover for one hour in ASW. The numbers of pulsations in one minute at baseline before exposure to gentamicin were then compared to pulsations/minute after transfer to ASW.

Results: Ephyrae were paralyzed during 1mM gentamicin exposure, but jellyfish exposed to up to 48 hours of gentamicin recovered 92% of pulsatile function.

Conclusions: 1 mM gentamicin is strong enough to paralyze ephyrae, but toxicity is, in fact, fully reversible. *A. aurita* is a positive animal model on which to study human ototoxicity.

APPROVED BY DIRECTOR OF HONORS THESIS

_____

Dr. Rizalia Klausmeyer, Department of Chemistry

APPROVED BY THE HONORS PROGRAM:

_____

Dr. Andew Wisely, Director

DATE: _____

REVERSIBILITY OF AMINOGLYCOSIDE INDUCED OTOTOXICITY IN JELLYFISH EPHYRAE

A Thesis Submitted to the Faculty of

Baylor University

In Partial Fulfillment of the Requirements for the

Honors Program

By

Akansha Mohan

Waco, Texas

December 2013

TABLE OF CONTENTS

CHAPTER ONE

Background

Anatomy of the Ear

The ear consists of three parts, the outer ear, the middle ear, and the inner ear. The outer ear and the middle ear are responsible for transmitting sound to the inner ear. The inner ear transduces vibrations of the perilymph into nervous impulses that are transmitted further to the auditory lobe. The inner ear consists of two labyrinths. The bony labyrinth lies within the temporal bone and the membranous labyrinth lies within the bony labyrinth. The inner ear derives from an auditory vesicle of ectodermal origin from the embryo's head, and the vesicle eventually forms a utricle and a saccule. The utricle and saccule lie in a central part of the temporal bone, called the vestibule, and the utricle branches into three semicircular canals and the saccule proceeds on to form the cochlea.

The cochlea is responsible for hearing, and the vestibular apparatus is responsible for balance. The cochlea and vestibular apparati have different functions, but they morphologically and functionally both operate by hair cells. Hair cells are specialized sensory mechanoreceptors which are called hair cells because they contain a bundle of stereocilia that contain actin and which sense fluid movement in cavities of the inner ear. Hair cells in the cochlea are innervated by the cochlear nerve. Hair cells of the vestibular apparatus are located in the utricle and saccule in sensory epithelium of the macula

located on swellings called ampullae at the base of the semicircular canals. The macula's neuroepithelial cells are innervated by the vestibular nerve.

Anatomy and function of the cochlea[1]

The cochlea is the main auditory part of the inner ear. It runs as an anterior to posterior coiled loop which is wider at its base similar to a conch shell, containing about three turns. It contains three fluid filled cavities, including, from top to bottom, scala tympani, scala media, and the scala vestibuli. The scala tympani and scala vestibuli both contain a fluid called perilymph, which contains a high concentration of potassium ions and low concentration of sodium ions, and is thus similar in composition to cerebrospinal fluid. The scala media contains endolymph, which, in contrast to perilymph, has a high concentration of sodium ions and a low concentration of potassium ions. For sound transmission, the vibration of tympanic membrane leads to successive vibrations of the three middle ear ossicles, the foot plate of the stapes in the oval window, the perilymph in the scala vestibule, and finally vibrations of the basilar membrane. At its basal end, the basilar membrane is stiff but gradually becomes wider and more flexible. Higher frequency sounds displace the basilar membrane near the oval window, and lower frequency sounds displace the basilar membrane further away from the oval window. The Organ of Corti lies inside the cochlea and has hair cells characterized by "hair" or stereocilia. The cells are attached at their base on the basilar membrane, and the tips of the cell fuse with the tectorial membrane. As sound waves vibrate the basilar membrane, a shearing force between the basilar membrane and the tectorial membrane develops, and the stereocilia are bent back and forth which leads to depolarization in hair cells and

creation of electrical signals. Hair cells are of two types: the outer hair cells (OHC) and the inner hair cells (IHC). Both types of cells consist of stereocilia which are shorter near the modiolus and taller laterally. While both types have afferent and efferent endings, IHC have more afferent endings. OHC amplify the vibrations, and the IHC convert the mechanical signald via ion channels into electrical signals. Auditory nerve fibers rest below the hair cells and pass these signals on to the brain.

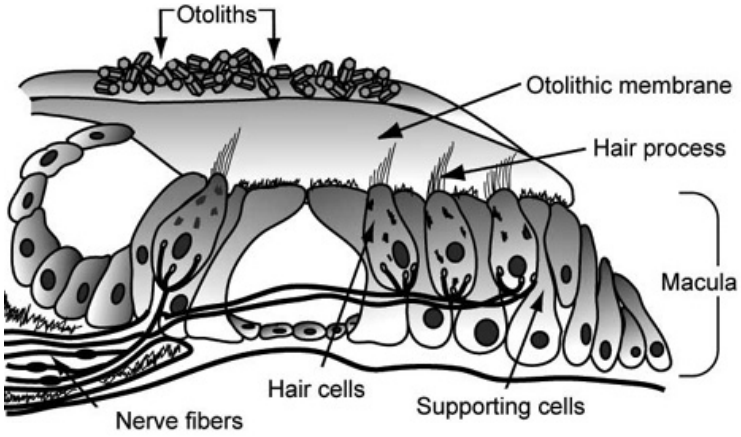


Figure 1: Vestibular Apparatus[2]

Anatomy and Function of the Vestibular Apparatus

In the vestibule, neuroepithelial cells line the saccule and the utricle; these cells detect linear acceleration. These cells are classified as "hair cells" because of their stereocilia. Stereocilia are covered by a gelatinous layer that contain calcium carbonate crystals called "otoliths" that change position based on head movements related to gravity. The shearing of the hair cells against the gelatinous layer can cause deformation of the hair cells, resulting in action potentials that that are carried via the vestibular branch of the eighth cranial nerve. Similarly, the semicircular canals have receptor areas in their ampullae called cristae ampullaris, which detect angular acceleration. Cristae are

also covered with a gelatinous layer, called the cupula. Movement of the cupula over the cristae leads to bending of stereocilia and generation of action potentials. Unlike cochlear hair cells, each vestibular hair cell consists of a bundle of 50-100 stereocilia and a single kinocilium.[3] From the vestibular apparatus, signals go to neurons that control eye movements in the Vestibulo-ocular reflex (VOR) and to the cortex for coordinating body muscles for balance.

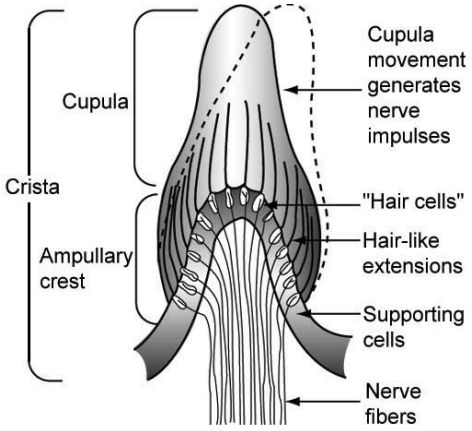


Figure 2: Crista[2]

Aminoglycoside Ototoxicity

Commonly used medications which lead to sensorineural hearing loss (mostly a result of hair cell loss) include aminoglycoside antibiotics, platinum-based anti-cancer medications of which Cis-platinum is the lead example, loop diuretics, macrolide antibiotics, and antimalarial medications.[4] Other common causes of hair cell loss related sensorineural deafness are aging (presbycusis) and exposure to intense noise. This review

is limited to the ototoxicity by aminoglycosides. Mechanism of damage by Cis-platinum is similar to that of aminoglycosides, and involves the formation of free radicals.[5]

Aminoglycoside[6] antibiotics include gentamicin, a prototype aminoglycoside (used in our research for that reason), tobramycin, kanamicin, neomycin, and amikacin. They are obtained either from actinomycetes in the soil, which are a kind of gram-positive bacteria, or from their synthetic derivatives. They inhibit protein synthesis by selectively targeting prokaryotic ribosomes and thus do not affect eukaryotic cells.

Aminoglycoside antibiotics, including gentamicin, are sometimes the best option to treat acute gram-negative infections, and they are most often used to treat bone infections, urine infections, and other recurrent pseudomonas lung infections in patients with cystic fibrosis. They are a second-line antibiotic for tuberculosis, which affects almost a third of the world's population. Aminoglycosids are reemerging as a choice in anti-tubercular medications as resistance to first-line antibiotics increases.[7] Aminoglycosides can be produced at very low cost, making them very attractive to populations where these infections commonly spread as life-threatening epidemics. Other advantages to aminoglycosides are that they are (1) hypoallergenic, (2) more bactericidal than many other antibiotics, and (3) have less antibiotic resistance such as is seen commonly in fluoroquinoloes, which are another class of antibiotics used to treat gram negative infections. Although aminoglycosides are such highly valuable antibiotics, their use is forcibly declined because of their severe ototoxicity and nephrotoxicity. The mechanisms by which aminoglycosides cause oto- and nephro-toxicity are strikingly similar.[5]

Approximately one third of patients who use aminoglycoside antibiotics for acute infections experience at least some degree of hearing loss, and about 15% patients who use aminoglycosides for acute infections experience some degree of vestibular symptoms.[8] In patients with tuberclosis who used aminoglycoside antibiotics for six to twelve months, some degree of hearing loss occured in all patients.[7] Aminoglycoside antibiotics differ in their degree of toxicity; neomycin is the most toxic, followed by gentamicin, kanamycin, tobramycin, amikacin, and netimicin. Amikacin, neomycin and streptomycin are more likely to damage the cochlea, and gentamicin and streptomycin are more likely to affect the vestibular hair cells.[9]

The mechanism of aminoglycoside ototoxicity has been researched extensively. As evidenced by fluorescent tags, aminoglycosides first enter the endolymph from the blood and then affect hair cells. They accumulate in inner ear cavities and persist even after cleared from the serum.[10] The antibiotic enters hair cells through nonselective channels of stereocilia although precise receptor mechanisms remain unclear.[11] Some researchers postulate that the cationic portions of the aminoglycoside interact with the anionic portions of the hair cells, which results in their rapid transport into the hair cells.[12] Cationic portions accumulate inside lysosomes, similar to their action in tubular cells in the kidneys.[13]. Other researchers present evidence that mutant mice which do not express the gene Myosin VIIIA are protected against aminoglycoside ototoxicity, suggesting that proteins which are expressed by Myosin VIIIA facilitate aminoglycoside ototoxicity. The most convincing evidence, however, suggests that the main mechanism of damage is mediated through aminoglycoside reactions with transition metals, especially iron, to form free radicals or Reactive Oxygen Species (ROS) which cause hair

cell apoptosis or necrosis.[14, 15,16] In one study, aspirin decreased hair cell loss by 75%,[17] presumably because salicylates c.helate ions are free radical scavengers. Vestibular toxicity is also mediated by ROS.[18]

Impact of Senorineural Hearing Loss (SNHL) on Quality of Life

Almost 15% of U.S. adults have decreased hearing. [19] SNHL associated with hair cell loss is the most common reason. Because hair cell loss is irreplaceable in humans and all mammals in general, most cases of deafness involve permanent deafness. Patients who do not hear well experience feelings of alienation and depression. [20] Although hearing aids and cochlear implants can help, loss of hearing affects people severely. Ototoxic drugs and aging also lead to loss of hair cells in the vestibule, which is also a permanent loss. Affected patients have dizziness, poor balance, or ataxia, abnormal eye movements (nystagmus), and often nausea and vomiting as a result of the unsteadiness and sensation of spinning. The vestibule-ocular reflex (VOR) is the most important vestibular pathway and is affected bilaterally by aminoglycosides which enter through the endolymph from bodily serum. Patients with their VOR negatively impacted by aminoglycosides suffer most commonly from oscillopsia. Vestibular dysfunction causes significant emotional distress, frequent hospitalizations, and can even be financially taxing due to difficulties with driving and employment.[21]

Patients with loss of hair cells have few ameliorating options. As yet, regeneration of human hair cells has not been possible.[22] Animal research, however, is underway to find drugs that can trigger hair cell regeneration in the human ear. In January 2013, Harvard researchers showed, for the first time, that regeneration of hair cells can occur in

mammals, specifically in a murine model. To trigger regeneration of these hair cells, patients used a drug that inhibits a protein called Notch which inhibits regeneration.[23] Further investigation concerning Notch remains.

Until sensorineural hearing loss can be treated in humans, one of the options for severely deaf patients is to consider surgical placement of cochlear implants[24]. These mechanical devices work by acting directly on cochlear nerves using electrical impulses instead of using the usual basilar membrane deflections as a stimulus. Although hearing is improved by such surgical replacements, patients also ultimately are at a risk for meningitis 30 times more than the general population. Additionally, the device and implantation of the implant can cost over $40,000. For these reasons, better alternatives to cochlear implants are being researched.

Viability of Aurelia aurita as an Animal Model for Ototoxicity

Aurelia aurita are transparent jellyfish that belong to the philum Cnidaria.[25] [26] Cindarians are aquatic animals mostly found in marine waters. They include jellyfish, hydras, sea anemones, corals, and the Portuguese man-of-war. They all have stinging cells called cnidocytes, and the name "Cnidarian" literally means "stinging creature" in Latin. *Aurelia* are commonly referred to as moon jellyfish, which are the most common scyphozoan jelly fish, and they are commonly found near North American shores, typically at about a depth of 20 meters. Cnidarians are carnivores; smaller jellyfish eat zooplankton and bigger jellyfish can also eat crustaceans. They are invertebrates with radial symmetry and three layers: an ectoderm, mesoglea, and endoderm.

Cnidarians change their form in alternate generations. [27] [28] The adult sexual generation is a form referred to as a medusa. This has the well-known appearance of a typical jellyfish with an upper umbrella and lower tentacles facing downwards. The upper surface of the umbrella is smooth and is called exumbrella and the undersurface is referred to as the subumbrella, which has an orifice that serves as both mouth and anus of the jellyfish. This undersurface has a membrane called the velum to which are attached the tentacles and at its center is the mouth which leads to a gastrovascular cavity. Medusa are either male with sperm producing gonads or females with ova producing gonads. Their mating and sexual reproduction leads to production of eggs, which hatch and lead to production of tiny oval hairy and short-lived structures called planulae that eventually attach to a substrate to become polyps. Polyps are the alternate generation to medusa, and jellyfish spend more time in the polyp generation than in the medusa generation. [29] In contrast to medusa, polyps are sedentary or sessile with tentacles facing up and are cylindrical forms that attach themselves to solid substrates such as rocks. This appearance is typically seen in coral reef. They grow by budding, and they attach to each other to form large colonies. Polyps reproduce asexually by cloning in a process called strobilation. In strobilation, the stalk portion of the polyp develops transverse grooves leading to a stack of discs appearance. Each of these discs proceeds to bud off into tiny baby jellyfish called ephyrae, the larval stage of the jellyfish.[30] The ephyrae eventually become medusa, marking a return to the sexual generation. The extent of strobilation is variable, but healthier polyps will give rise to more ephyrae more quickly. Monodisc stobilation generates a singly ephya per stobila, while polydisk strobilation produces up to 30 ephyrae.[31, 32] Ephyrae, which are about 4 to 15 mm in size grow to become the adult

(medusa) form of the jellyfish, which can have a breadth of several feet. Except for size, ephyrae and medusae are morphologically similar. [33]

Jellyfish have an advanced but non-centralized nervous system with nerves throughout their body, which is called a Nerve Net, and jellyfish use the nerve net to respond to the environment. This nerve net covers the subumbrella, but it is also distributed throughout the body so that the whole body of the jellyfish is stimulated in response to an insult anywhere on the body. These nerve impulses then accumulate in eight neuronal aggregations or ganglia called rhopalia that are found at regular intervals on the margin of the bell (the umbrella) in both the medusa and the ephyrae.[34] These ganglia are interconnected by longitudinal tracts across the velum on the subumbrella, and in a circular tract called inner nerve ring on the bell margin. Each rhopalia contains a gravity sensor by which jellyfish can perceive if they are floating up or down or are tilted. The rhopalia may also contain olfactory sensors, light sensors, and eyes. Each rhopalia has two ocelli that have the appearance of being photoreceptors.[35] Rhopalia aid jellyfish when they seek deeper water to avoid sunlight or if water currents are too strong as in stormy weather.

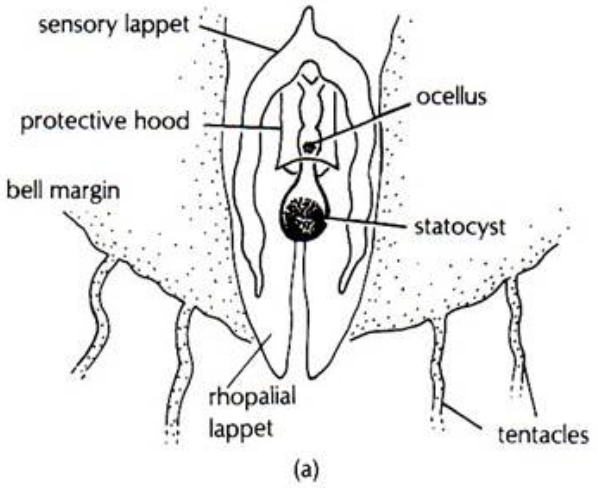


Figure 4: Jellyfish Rhopalia[36]

Cnidarians are the most primitive animals to show such neurological prowess, and they are more advanced than sponges in that they use very fast signal conduction to react with quickly directed behavior in response to complex sensory stimuli. They also exhibit complementary internally generated rhythmic behavior related to pulsatile swimming. Their nerve nets for feeding and movement appear independent, and the rhopalia have a regular rhythm to coordinate swimming and show pulsations even when isolated in vitro.[37]

Jellyfish have coronal, longitudinal, and radial muscles. Circular muscle contraction expels water from the subumbrellar cavity to propel the body forward, and the coronal muscle pulsates to help the animal move. The loose gelatinous mesoglea helps keeps jellyfish buoyant. They are capable of upwards movement, with quick upwards pulsations followed by a slow sinking in which they spread tentacles again to maximize catching prey.[38] The swimming is like the opening and closing of an umbrella,

in which the opening collects the water in the subumbrellar cavity and its closing propels the animal in the opposite direction. The direction of swimming depends on location of food, threat, or other environmental factors such as light and temperature. One review concluded that Aurelia's "swimming in response to somatosensory stimulation, swimming down in response to salinity, diving in response to turbulence, avoiding rock walls" are not simple reflexes but require specialized sensory receptors to provide feedback to directed behavior.[39]

Although rhopalia coordinate jellyfish motor movements by directing muscle contraction, sensory information is provided to the system by clusters of hair cells which are present on the velum, the membrane of the subumbrellar surface, and on the bases of tentacles.[40] Mechanoreceptors associated with the cnidocytes of Aurelia send impulses through axons which run through the jellyfish's central nervous system.[41] Mechanical currents near these hair cells lead to evoked potentials which are transducted intracellularly. Potentials transmitted intracellulary from the nerve ring were actually able to be recorded in Aglathia, another species of jellyfish.

The jellyfish hair cells is remarkably similar to the human inner ear hair cell and is also extremely sensitive to changes in position and environment. Hair cells are directionally sensitive, and morphological polarization of the hair cell bundle in vertebrates is related to this functional directionality.[42] Deflections of the hair bundle towards its long end evoke depolarizations, while deflections in the opposite direction produce hyperpolarizations; deflections at right angles to this axis produce no change in membrane potential.[43] Such characteristics hint that the cnidarian hair cell must be evolutionarily related to the human hair cell.

Research has shown that hair cells in jellyfish and in vertebrates (including the human ear) are similar morphologically and functionally. Hair cells in jellyfish have a central non-motile cilium surrounded by a collar of microvilli.[40] The microvilli are graded in length, from long on one side to short on the other, giving the collar a marked polarity and the cilium is located at the long end. Such a hair bundle is amazingly also the central feature of the human hair cell. While jellyfish hair cells are simple and fewer, they share the 9+2 pattern of microtubules of ciliae in humans. In jellyfish, however, the stereocilium, however, does not appear to be involved in mechanoelectrical transduction, whereas in humans where they are vital to eventual signal transduction.[44]

The tactile hairs on the velum of jellyfishes are set in specific orientation suggesting that, like vertebrates, they too are mechanoreceptors that possess directional sensitivity. The velum is also a delicate membrane that vibrates on receiving mechanical currents, and its relationship to hair cells is similar to the relationship the basilar membrane holds in relation to hair cells in the organ of Corti.[40] While jellyfish hair cells are strictly sensory neurons, vertebrate hair cells are epithelial structures which each connect to an afferent neuron at their ends. The similar morphology and function predict that effects of toxins on hair cells of invertebrate jellyfish should mirror the effects of toxins on vertebrate hair cells, including those in the human ear. Additionally, since hair cells serve as primary sensors of orientation in jellyfish, impairments in behavioral swimming and pulsing abilities can be correlated to degree of damage to hair cells.

Existing Animal Models for Studies in Ototoxicity

Loss of hair cells is the most common reason of hearing loss, and hair cell loss is considered permanent in mammals. The only viable solution to permanent hair cell loss is to discover safe means by which hair calls in the human ear can be stimulated to regenerate. The inner ear, however, which houses the hair cells, is relatively inaccessible, and research in ototoxicity is made even more difficult because the changes that occur in dying hair cells are at a microscopic level. Additionally, molecular mechanisms that can be significant enough to influence hair cell regeneration are complex.

For over two decades, researchers have attempted to study ototoxicity in animal models. Scientists have studied the effectiveness of different animal models for human ototoxicity by examining behavioral responses to aminoglycosides and intense noise, impairment of responses to auditory brain-stem evoked potentials following aminoglycoside exposure, and the morphological changes that can occur in vitro on cell cultures of hair cells. Science has successfully regenerated hair cells in evolutionarily "lower" animals, such as birds, fish, and invertebrates, but mammalian animal models which both are sensitive to aminoglycoside exposure and are able to regenerate lost hair cells remain elusive. The remaining chapter discusses the three most commonly used animal models for aminoglycoside ototoxicity, the chicken, mouse, and zebrafish, and compares and contrasts them to this study's newly proposed jellyfish model for aminoglycoside ototoxicity.

Chickens. As mentioned before, hair cell regeneration was found to be common in birds, including chicken, quail, parakeets, and other birds, and indeed some scientists

posit birds as currently the most positive animal models we have for human otoxoxicity. Current research postulates that the hair cells are able to be regenerated because supporting cells develop into newer hair cells. [45] Strikingly, regeneration in auditory ephithelia is possible even after exposure to high-intensity sound, aminoglycosides, and Cis-platinum. Regeneration of hair cells in birds is promising because even though birds are not vertebrates, they are warm blooded animals, and thus closer to the vertebrate than fish. Just as hair cells in the chicken cochlea can regenerate, hair cells in vestibular epithelia can also be spontaneously regenerated, as evidenced in chicken vestibular apparati.[46] Limitations of using the bird as a viable animal positive model include of course expense, size, and limited reproducibility with long gestation periods.

Mice. Although mice and rats have the ability to undergo transgenic experimentation and can reproduce relatively easily, aminoglycoside ototoxicity was previously not studied in mice or rats because it was believed that they were resistant to these antibiotics. Neverthless, Wu et al in 2001 found that mice did in fact show ototoxic side effects, similar to humans, in response to aminoglycoside exposure. Aminglycoside exposure reduced the degree of brain stem evoked auditory responses, affected vestibular function, and led to a loss of both auditory and vestibular hair cells.[47] Mice also show impaired swimming ability, evidence of disordered vestibular dysfunction, after aminoglycoside exposure. Although avian regeneration gave hope that hair cell regeneration may occur in mammalian species, scientists failed to regenerate hair cells in the gerbil[48], and there was, in fact, no hair cell regeneration after embryonic day 14. [49]

Although a few cultured murine supporting cells do differentiate into hair cells in vitro, recovery from this damage was limited mainly to vestibular compensation. [50,51]

Nevertheless, in January 2013, scientists were able to regenerate, for the first time, lost hair cells in mice with the aid of a drug that inhibited Notch, an inhibitor of hair cell mitosis.[23] Conclusions of this study have yet to be tested on humans, and further research remains to establish mice as positive models for hair cell regeneration.

Zebrafish. Zebrafish have emerged recently as a possible model for aminoglycoside ototoxicity. An obvious limitation to using the zebrafish as a model for human ototoxicity is that zebrafish are cold blooded vertebraes. They do, however, have transparent inner ears, an easily accessible lateral line which is located outside their body, and mechanoreceptors exist in both locations. The mechnoreceptors are histologically and functionally similar to hair cells in the inner human ear. These hair cells help the zebrafish in sensing environmental stimuli such as water currents, prey, and danger and responding with change in direction and manner of swimming. Just as murine, avian, and human hair cells, zebrafish hair cells are sensitive to aminoglycosides and Cis-platinum. The pattern of hair cell loss is also remarkably similar to the loss of hair cells in the organ of Corti, proceeding from the base to the apex of the cochlea, and from the outer hair cells to the inner hair cells. Moreover, just as sensorineural hearing loss in humans manifests as loss of hearing of higher frequency sounds, the zebrafish have fewer brain stem provoked auditory responses in responses to sounds of higher frequency.

Inhibiting Notch in zebrafish also regenerated hair cells, and at a remarkably rapid rate.[52,56] The similarity of human hair cells to zebrafish hair cells, combined with the ability to regenerate hair cells rapidly in zebrafish, has resulted in their being used already to test otoxtoxicity of several chemicals.[57]

Jellyfish. As discussed previously, jellyfish ephyrae can also serve as a positive animal model of ototoxicity. Jellyfish ephyrae possess hair cells that are similar to human hair cells in both morphology and polarity. They generate action potentials in response to minor mechanical displacements of the velum, which is very reminiscent of the basilar membranes in the human cochlea and vestibular epithelia. [40]

Using moon jellyfish *Aurelia*, and its larval stage ephyrae, as models for development of graviceptors were first pioneered by Dr. Sprangenberg starting in the 1960s. She noted that rhopalia of ephyrae developed statocysts with statoliths, ocelli, ciliated mechanoreceptors cells, and gravireceptors, all of which feature hair cells as their basic units. [53] Amazingly, the development of these rhopalia is so hardy that they emerge even in ephyrae grown in space.[54]

Like zebrafish, jellyfish can also reproduce quickly and reliably over long periods of time. Dr. Spangenberg's lab, for instance, features a polyp colony that was started in 1935 by Frank J. Lambert in Essex, England. [55]

Advantages of Using Aurelia as Animal Model for Ototoxicity. The zebrafish is the most important competitor for the jellyfish; birds are inconvenient animal models and

murine animals have yet to show reliable hair cell recovery. Aurelia possess four major advantages over zebrafish to be a positive model for aminoglycoside ototoxicty.

1. Movements of ephyrae can be studied immediately after their metamorphosis from polyps. At 12 to 21 degrees C, ephyrae increase in size from 4 mm to 1.4 cm over 14 days. Their small size is preferable to the relatively larger size of other animals, such as the jellyfish, which can grow up be about 6 cm in size.[56]

2. The zebrafish develops its nervous system slower than *A. aurita.* Complex behaviors such as responses to visual and auditory stimuli are only apparent from 5 days after fertilization.[57] Sexual maturity can take up to three months.[58] Perhaps most importantly, the semicircular canals are not functional until the zebrafish are a month old.[59]

3. *A. aurita* govern their movements almost entirely by mechanoreceptors since they have such a rudimentary vision system. Since zebrafish have tetrachromatic vision (ability to see ultraviolet light), which is actually even more advanced than human trichromatic vision, behavioral impairments due to aminoglycoside exposure may be mitigated by their spectacular ocular vision. [60] Degree of behavioral impairment in Aurelia will therefore correlate more closely with extent of vestibular hair cell impairment.

4. Jellyfish and other cnidarians use only jet propulsions, which can originate only from their nervous system. Aglantha, for instance, have a burst speed of 13 body lengths per second. [61] This is in contrast to fishes which propel with their tails and can use water currents. Thus, changes in swimming ability (an easily monitored behavioral

trait) will more likely and more reliably be indicative of nervous system dysfunction in jellyfish than in zebrafish.

Nevertheless, the zebrafish remain a very attractive model because of the extent of research that has been done on them. Zebrafish research results in one thousand publications a year. Neurological studies on *Aurelia* is limited to the studies Dr. Spangenberg has conducted in the past thirty years.

Previous Studies of Ototoxicity on Aurelia aurita

Recently, studies by other researchers at our laboratory have found a significant dose-dependent relationship in loss of hair cells both functionally and morphologically when gentamicin was used in doses of 1mM, 2mM, and 3.5mM. Specifically, morphological hair cell loss occurred in rhopalia of Aurelia ephyrae that were exposed to gentamicin at a 3.5mM.[62]

Research Objectives and Importance of Research

While it was apparent that jellyfish hair cells experience permanent histological damage when exposed to 3.5mM gentamicin after even a short duration period, it remains unclear if gentamicin exposure for a longer period at a smaller dose would also result in irreversible hair cell loss.

This study seeks to explore if 1 mM gentamicin is a strong enough dose to affect jellyfish hair cells, which are both functionally and morphologically similar to human hair cells, while still allowing for hair cell recovery and reversible toxicity. The possible

impacts of longer exposure to gentamicin were also tested. Jellyfish were exposed to 0, 1, 24, and 48 hours of gentamicin, and their pulsatile activity was recovered before and after their exposure to gentamicin. Pulsatile activity is linearly correlated to degree of functionality of hair cells.

The study has the potential to have long term implications. If toxicity is shown to be reversible in jellyfish, which have hair cells remarkably similar to mammalian hair cells, then gentamicin, an important and effective antibiotic, can perhaps be further investigated with the aim of eventually reintroducing it at a smaller dose over a longer period of time to human populations.

This study also aims to further study and evaluate the suitability of *Aurelia auritarita* as an efficient model for aminoglycoside ototoxicity.

CHAPTER TWO

Materials and Methods

Culturing Ephyrae

All experiments were conducted in the laboratory of Dr. Dorothy Sprangenberg at Eastern Virginia Medical School in Norfolk, Virginia. The lab collects *Aurelia auritarita* polyps yearly in Norfolk, Virginia , and they were cultured in artificially made sea water (ASW). Strobilation (metamorphosis) was induced by iodine which jellyfish use to synthesize jellyfish-thyroxine, or by thyroxine /1,2/. Iodine and thyroxine solutions were made up in ASW to achieve concentrations of 1×10^{5M} for induction of metamorphosis. The cultures were fed *Artemia salina* (brine shrimp) once weekly and transferred to artificial sea water in clean culture dishes pre-treated with anion-exchange beads to remove iodide. Within 72 hours of inducing strobilation at 28C, ephrae develop eight ganglion like rhopalia and the ability to swim and balance themselves similar to medusa jellyfish. The rhopalia give rise to mechanoreceptor cells("hair cells")[63]. Very similar hair cells have been found in other animals, including vertebrates[64]. *Aurelia auritarelia* use these cells to sense gravity and correct orientation.[65] Rhopalia also stimulate spontaneous rhythm of the animal's pulse, and the connections of the rhopalia with the giant fiber nerve net conduct excitation at each beat. The giant fibers then propagate the contraction wave over both radial and circular muscles.

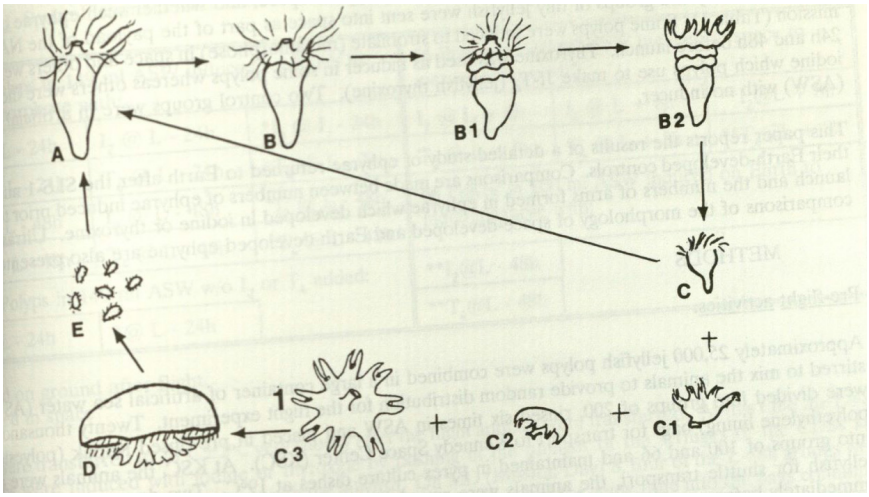


Figure 5: Life Cycle of Laboratory-Grown *Aurelia auritarita* (not drawn to size)
Representations
A. Polyp which self-replicates by budding
B. Strobilation stages (B and B1 represent early stages, B2 represents a late stage showing ephrae formation)
C. Post strobilation stages (C represents free-swimming ephyrae)
D. Medusa
E. Planular larvae

Experimental Procedure

Once strobilation and growth of ephyrae is complete, the procedure consists of three parts:

1. Preparing test tubes for experimentation and selecting "Swimmer" jellyfish.
2. Preparing 1mM gentamicin solutions.
3. Carrying out three comparative experiments involving 40 jellyfish each. Each of the jellyfish were exposed to varying duration times of gentamicin ("experimentation").

These are described in more detail below.

Preparing test tubes for Experimentation and Selecting Swimmer Jellyfish

1. 40 flat bottom, 15 ml glass test tubes were placed in a test tube rack. These were the "first set, or priming set" of 40 test tubes.

2. 10 ml of artificial sea water were put in each of the forty test tubes using a pipette.

3. Single ephyrae were pipetted from the culturing dish to each of the forty test tubes.

4. Baseline behavior of ephyrae was observed and recorded for each of the ephyrae. If any of the ephyrae were not swimmers, they were replaced such that prior to starting the experiment all 40 ephyrae were confirmed swimmers.

5. An ephyra is confirmed as a swimmer by the following two criteria:

    a) Swimmers will be able to consecutively pulse four times in a specific direction.

    b) They will exhibit their normal orientation in which the ephyra's mouth will point downwards.

As explained above, the aforementioned procedure occurred three times to provide for three comparative experiments.

Preparation of Gentamicin Solutions

1mM gentamicin solutions were to be prepared.

238.79 mg of commercially available Gentamicin Sulphate salt powder (477.59 g/mol; Sigma-Aldrich, St. Louis, MO) were put in in 500 ml of ASW and placed in big flask. This flask was stored in the refrigerator at 4 degrees C. This was repeated two more times.

The three resulting identical 1 mM gentamicin solutions were used in experimentation.

Experimentation

1. A set of 40 new, rinsed and dried, and numbered test tubes were placed in a test tube rack. Each was filled with approximately 10 mL of artificial sea water.
2. 40 ephyrae that were confirmed swimmers (see above) were pipetted into the set of these 40 numbered test tubes.
3. The number of pulsations per minute in ASW were recorded ("baseline data").
4. A second set of 40 new, rinsed and dried, and numbered test tubes were prepared. Test tubes 1-10 were filled with 10 mL of ASW, and test tubes 11-40 were filled with 10 mL of 1mM gentamicin.
5. Jellyfish ephyrae were transferred from the first set of test tubes to the corresponding test tubes in the second set. Thus, jellyfish in test tubes 11-40 are transferred from ASW to 1 mM gentamicin, while jellyfish in test tubes 1-10 serve as controls (transfer from ASW to ASW).

6. After transfer to the second (test) set of test tubes, ephyrae were kept in these test tubes for varying durations of time. Trial 1 held the 40 jellyfish in the second set of test tubes for one hour, Trial 2 held the jellyfish in the second set of test tubes for 24 hours, and Trial 3 held the jellyfish in the second set of jellyfish for 48 hours. They were placed in incubators.

7. The number of pulsations per minute were recorded for each jellyfish at set times while they were incubated. Observations were taken at 1 hour, 24 hours, and 48 hours during incubation. (Since Trial 1 held the jellyfish in incubation for only 1 hour, there is no incubation data for trial 1, only baseline and recovery data).

8. A third set of 40 new, rinsed and dried, and numbered test tubes were prepared. 10 mL of ASW were placed in each of these test tubes.

9. Upon completion of incubation time, the 40 jellyfish were pipetted carefully yet again into the third set of test tubes, each of which were filled with artificial sea water.

10. After one hour in artificial sea water, the number of pulsations per minute were recorded for each jellyfish ("recovery data").

CHAPTER THREE

Results

Hypotheses

Null Hypothesis: There is no difference in the mean percentages of recovery of pulsatile motion after an hour in artificial sea water among groups of jellyfish exposed to varying durations of exposure times to gentamicin.

Alternate Hypothesis: The mean percentages of recovery of pulsatile motion after an hour in artificial sea water will be less for those groups of jellyfish exposed to gentamicin for a longer period of time.

Four groups of Jellyfish were tested at baseline and after 1 hour of recovery time in artificial sea water (ASW) after being exposed to gentamicin for varying durations of time.

a. Group 1- *Jellyfish 1-10*: No exposure to gentamicin
b. Group 2-*Jellyfish 11-20*: 1 hour of exposure to gentamicin
c. Group 3-*Jellyfish 21-30*: 24 hours of exposure to gentamicin
d. Group 4-*Jellyfish 31-40*: 48 hours of exposure to gentamicin

ANOVA (Analysis of Variance)

A successful ANOVA depends on sample data meeting three assumptions, listed below. The experimental data successfully fulfilled all three assumptions.

1. *Independently collected samples.*

2. *Normality.* All groups show a normal distribution, as tested by the Normal Distribution Function of Excel.

3. *Homoscedasticity (equality of variances).* There is an inequality in variance values, and some samples show a relatively large degree of variance (below). This can be reversed in future tests by primarily testing a larger sample size. Inequality of variances, however, can also possibly reflect an inherent weakness of the jellyfish model for aminoglycoside ototoxcity.

Data

Table 1: Mean Number of Pulses at Baseline in ASW *Before* Treatment ("Baseline Data") and *After* One Hour of Recovery in ASW ("Recovery Data")

JF #	Group 1 Before	Group 1 After	Group 2 Before	Group 2 After	Group 3 Before	Group 3 After	Group 4 Before	Group 4 After
1	27	20	116	100	21	36	11	19
2	44	39	33	19	73	40	46	36
3	21	35	46	22	70	54	25	54
4	25	75	35	31	42	38	28	62
5	51	54	70	70	32	72	52	45
6	20	34	54	49	45	27	121	46
7	81	38	10	6	44	21	25	60
8	26	16	62	51	43	56	42	26
9	37	46	19	30	42	26	42	47

10	44	15	31	33	41	49	26	36
11	19	LOST	45	42	40	21	11	28
12	48	36	36	19	40	33	68	20
13	45	63	28	22	39	25	55	35
14	70	30	35	27	38	47	12	12
15	33	60	25	33	37	39	24	6
16	40	76	27	8	36	36	22	18
17	87	95	76	18	35	26	16	12
18	17	22	41	24	34	22	54	19
19	26	33	67	20	33	28	28	16
20	21	53	29	20	32	34	22	10
21	9	12	30	20	31	40	30	30
22	24	16	17	3	30	28	14	25
23	62	44	42	LOST	85	31	16	17
24	20	22	62	15	66	12	20	10
25	10	10	34	46	38	62	24	26
26	35	42	30	12	32	24	42	21
27	34	66	30	29	11	11	36	39
28	22	21	43	36	81	86	17	33
29	41	28	74	18	67	55	33	28
30	91	54	18	22	29	35	16	17

Table 2: Percentile Recovery of Pulsations/Minute

	Group 1	Group 2	Group 3	Group 4
1	0.740741	0.862069	1.714286	1.727273
2	0.886364	0.575758	0.547945	0.782609
3	1.666667	0.478261	0.771429	OUTLIER
4	OUTLIER	0.885714	0.904762	2.214286
5	1.058824	1	OUTLIER	0.865385
6	1.7	0.907407	0.6	0.380165
7	0.469136	0.6	0.477273	OUTLIER
8	0.615385	0.822581	1.302326	0.619048
9	1.243243	1.578947	0.619048	1.119048
10	0.340909	1.064516	1.195122	1.384615
11	LOST	0.933333	0.525	1
12	0.75	0.527778	0.825	0.294118
13	1.4	0.785714	0.641026	0.636364
14	0.428571	0.771429	1.236842	1
15	1.818182	1.32	1.054054	0.25
16	1.9	0.296296	1	0.818182
17	1.091954	0.236842	0.742857	0.75
18	1.294118	0.585366	0.647059	0.351852
19	1.269231	0.298507	0.848485	0.571429
20	2.52381	0.689655	1.0625	0.454545
21	1.333333	0.666667	1.290323	1
22	0.6875	0.176471	0.933333	1.785714
23	0.354839	LOST	0.364706	1.0625
24	1.1	0.241935	OUTLIER	0.5
25	1	1.352941	1.631579	1.083333
26	1.2	0.4	0.75	0.5

27	1.941176	0.966667	1	1.083333
28	0.954545	0.837209	1.061728	1.941176
29	0.682927	0.243243	0.820896	0.848485
30	0.593407	1.222222	1.206897	1.0625

*Irregular and nonavailable data points were eliminated. Box plots revealed that jellyfish ephyrae which either recovered less than 50% in pulsatile function after treatment (percentile recover <.5) or doubled (percentile recovery>2) classify as outliers. Only 4 of 120 points classified as outliers.

N.B. A percentile recovery value over one signifies an increase in the number of pulsations after the treatment (or no treatment, in the case of controls) as compared to baseline (before treatment), and a percentile recovery value under one signifies a decrease in the number of pulsations after treatment from baseline.

Table 3: Incubation Data

	1 HOUR			12 HOURS			24 HOURS		
JF #	P	S	O	P	S	O	P	S	O
11	0	NS	O-						
12	0	NS	O-						
13	0	NS	O-						
14	2	NS	O-						
15	0	NS	O+						
16	0	NS	O-						
17	0	NS	O-						
18	0	NS	O-						
19	0	NS	O+						
20	0	NS	O-						
21	0	NS	O-	0	NS	O-			
22	0	NS	O+	0	NS	O-			
23	0	NS	O-	0	NS	O+			
24	0	NS	O+	0	NS	O+			
25	1	NS	O+	0	NS	O+			
26	0	NS	O+	0	NS	O-			

27	0	NS	O+	0	NS	O-			
28	0	NS	O-	0	NS	O+			
29	0	NS	O-	0	NS	O-			
30	0	NS	O+	0	NS	O-			
31	0	NS	O-	0	NS	O-	0	NS	O-
32	0	NS	O-	0	NS	O-	0	NS	O-
33	0	NS	O-	0	NS	O+	0	NS	O+
34	0	NS	O-	0	NS	O-	0	NS	O-
35	0	NS	O-	0	NS	O-	0	NS	O-
36	0	NS	O-	0	NS	O-	0	NS	O-
37	0	NS	O-	0	NS	O-	0	NS	O-
38	0	NS	O-	0	NS	O+	0	NS	O-
39	0	NS	O-	0	NS	O-	0	NS	O-
40	0	NS	O-	0	NS	O-	0	NS	O+

P= Pulsations/Minute
S= Swimmer (Pulse 4 times in one direction); NS= NonSwimmer
O+= Correct Orienting (manubrium faces down); O-=Incorrect Orienting .

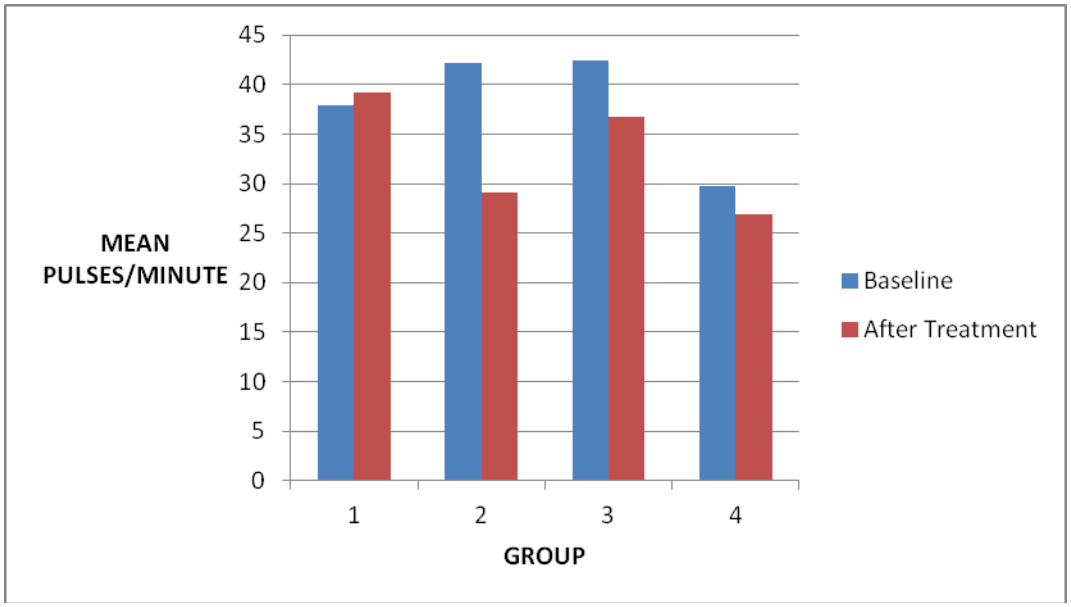
Jellyfish 11-20 are from Group 2 (1 hour of gentamicin exposure), Jellyfish 21-30 are from Group 3 (24 hours of gentamicin exposure), and Jellyfish 31-40 are from Group 4 (48 hours of gentamicin exposure).

Table 3 only includes data of jellyfish from test run 1, but subsequent tests showed nearly identical results. The complete loss of motor function of the jellyfish while in gentamicin is evident.

Table 4: Summary Statistics

Group	Mean Percentile Recovery (Pulses/Minute)	Variance
1 (Controls)	1.179415	.435275
2 (1 hour exposure to gent.)	0.735432	.134212
3 (24 hour exposure to gent.)	0.927179	.1111145
4 (48 hour exposure to gent.)	0.920767	.254335

Figure 1: Mean Pulsations/Minute Before and After Treatment



Mathematical Interpretation of Results

A one way ANOVA (Analysis of Variance) was employed to test if the mean number of pulsations before and after treatment was different among groups. The one way ANOVA was run by means of Excel.

A between groups p-value reflects the probability not due to chance that the null hypothesis is true (i.e, there is no difference in the mean number of pulsations after one hour of recovery in artificial sea water among groups of jellyfish exposed to gentamicin for varying amounts of time).

Four of the 120 jellyfish that we tested had a much lower recovery after exposure to gentamicin. We termed these jellyfish as outliers. Although the post-gentamicin recovery of jellyfish was complete, and was statistically similar after exposure to either

24h (group 3) or 48h (group 4) of gentamicin, our statistical comparison of recovery between groups 3 and 4 was done after exclusion of these outliers.

Groups 3 and 4 (exposure to gentamicin for 24 hours and 48 hours, respectively) yielded a p value= .922792. In other words, *there is a 92.3% probability (not due to chance) that the percentile recovery of pulsatile function from baseline for the jellyfish exposed to gentamicin for only 24 hours is no different from jellyfish exposed to gentamicin for 48 hours. In other words, hair cells recovered up to almost 100% functionality, even after gentamicin exposure.* The null hypothesis is supported.

The average percentile recovery of the controls is nearly 23% more than groups exposed to gentamicin for 24 or 48 hours (as expected). As a result, the one way ANOVA comparing all the samples yielded a p value of only 0.021786, reflecting that a significant difference was observed in recovery rates among group 1 (controls), group 2, and groups 3 and 4. Interestingly, Group 2 jellyfish (exposed to gentamicin for only one hour) showed the poorest percentile recovery, of only about 70%. On average, of all of the groups exposed to any duration of gentamicin (groups 2-4), there was, on average, an 85% return to baseline motor function after exposure.

CHAPTER FOUR

Discussion

Findings

Sensorineural hearing loss (SNHL) occurs commonly and is permanent. The National Health Interview Survey found that 37 million (1 in 10 Americans) report hearing loss.[66] 20 to 26 million of these Americans have sensorineural hearing loss, and 40% of individuals older than age 65 have sensorineural hearing loss. Hearing loss has the potential to affect physical, cognitive, behavioral and social function as well as quality of life, and research has linked SNHL to depression and dementia.[67] Because no cure exists for SNHL, the emphasis is on prevention.

SNHL and vestibular ototoxicity are almost always caused by degeneration of hair cells in the auditory and vestibular epithelium, and such loss is considered permanent because lost hair cells cannot be regenerated in mammals.[68] Hair cell loss occurs most often as a result of aging (presbycusis) and noise exposure, but also commonly because of ototoxicity to aminoglycoside antibiotics such as gentamicin.[69] A review of aminoglycoside induced ototoxicity concluded that such toxicity leads to permanent bilaterally severe, high-frequency SNHL and vestibular hypofunction, and that the permanent hearing loss is accompanied by degeneration of hair cells and neurons in the cochlea.[6] Other reviews have concluded that the permanent hearing loss caused by aminoglycosides is predominantly associated with the apoptotic death of outer hair

cells,[70] and that aminoglycosides generate free radicals within the inner ear, with subsequent permanent damage to sensory cells and neurons, resulting in permanent hearing loss.[71]

Although use of aminoglycosides has become severely limited because of their nephrotoxic and ototoxic side effects, aminoglycosides are very useful antibiotics that are exceedingly bactericidal and are also inexpensive and hypoallergenic. Research objectives accordingly focused on determining whether gentamicin can be administered at a dose strong enough to have therapeutic benefit with negative ototoxic side effects. Another objective of the research was to evaluate if *Aurelia auritarelita* jellyfish ephyrae can appropriately model not only aminoglycoside induced ototoxicity, but potentially also other SNHL induced dysfunction including presbycusis and noise-related SNHL.

Current literature shows that permanent aminoglycoside related SNHL and vestibular toxicity occurs even in low doses of aminoglycosides. In children, gentamicin led to irreversible ototoxicity even in patients without abnormal serum gentamicin levels.[72] Similarly vestibular toxicity, with associated permanent loss of balance, occurred after gentamicin at any dose, in any regimen, and at any serum level.[73] Another review concluded that gentamicin can cause permanent vestibular and auditory ototoxicity and that there is no safe dose of gentamicin. [74] In jellyfish ephyrae, as mentioned before, a 3.5 mM dose of gentamicin will result in permanent histological impairement of jellyfish hair cells.

The results of this study showed that 1 mM of gentamicin is strong enough to have a paralyzing impact on jellyfish, but that jellyfish hair cells were not irreversibly damaged, as evidenced by an almost 100% recovery to baseline pulsing ability.

During exposure to gentamicin for any length of time, all jellyfish were fully paralyzed and lost all movement. This indicates that the concentration of gentamicin used in the experiments was sufficiently high to exhibit toxicity. Jellyfish almost immediately lost all function while in gentamicin and for the entire duration of their time in gentamicin (i.e, jellyfish in gentamicin for 48 hours were paralyzed for the full 48 hours). Such a complete reaction to the gentamicin is evidence that the gentamicin dose used was not minimal and will plausibly have some therapeutic benefit when applied to humans.

Following exposure to gentamicin, jellyfish recovered almost completely after only one hour in artificial sea water. Surprisingly, jellyfish that were exposed to gentamicin for 48 hours showed no more adverse effects than jellyfish which were exposed to gentamicin for 24 hours. This is promising in that it suggests that administering gentamicin over longer periods of time will not result in more irreversible damage. In other words, if this data can be extrapolated to humans, exposing human hair cells to 1 mM gentamicin for even up to 48 hours, and potentially even longer, will lead to no permanent ototoxic damage.

This study is supported by other studies which have found that the extent of damage to hair cells is indeed dose dependent in both the cochlea and the vestibule. At low doses of aminoglycosides like gentamicin, hair cell loss is scattered, but higher doses of gentamicin result in loss of all hair cells. [75] Low doses will most likely also affect only

outer hair cells, and of the hair cells they do affect, they will only affect hair cells which reside near the basilar papilla. Higher doses have the potential to not only affect hair cells further, but to affect both type 1 and type 2 hair cells.[76]

Limitations

Our data has two minor limitations and must be interpreted with attention to these limitations. Jellyfish in group 2, which were exposed to gentamicin for only one hour, showed significantly lower recovery of pulsatile motion. The significance of this observation is unclear. The jellyfish in this group were transferred from artificial sea water to gentamicin and back to artificial sea water in a shorter period of time than the other groups, and the quick transfer process may have become too taxing. While other groups had transfers separated by 24 hours or 48 hours, this group's transfer to and from gentamicin took place in one hour.

As noted in chapter three, there was also an inequality of variances in the data. This can be alleviated in future studies by increasing the sample size. Nevertheless, the large degree of variance can also point to an inherent weakness of the jellyfish model. For instance, there is a large degree of variance in the baseline pulsations/minute per jellyfish.

Future Directions

Jellyfish ephyrae efficiently model the maximal dose and duration of gentamicin exposure that will not lead to lasting functional and morphological damage to hair cells. This experiment linearly correlated pulsatile ability to functionality and intactness of the

jellyfish hair cell. Further experiments can track histologic changes that may occur after exposing jellyfish to gentamicin to evaluate if the hair cells recover as well microscopically as they do functionally after gentamicin exposure.

Since gentamicin ototoxicity has been shown to be reversible in jellyfish ephyrae, which feature hair cells very similar to mammalian hair cells, future studies can aim to further understanding of gentamicin ototoxicity and nephrotoxicity, with the eventual hope of reintroducing gentamicin to human populations at smaller doses over longer periods of time.

REFERENCES

[1] Junaueira LC, Carneiro J . 2005. Basic histology. 11th ed. New York (NY): McGraw-Hill. pp 469-474

[2] Gardner A. Equilibrium.[online] Available from: http://virtualgardneranatphys.wikispaces.com/Equilibrium . Accessed on: 10/02/1013

[3] Purves D, Augustine GJ, Fitzpatrick D, et al. 2001. Vestibular Hair Cells. In: Neuroscience, 5th ed. Sunderland (MA): Sinauer Associates, pp 305-307

[4] Arslan E, Orzan E, Santraelli R. 1999. Global problem of drug-induced hearing loss. Annals of the New York Academy of Sciences 884:1-14

[5] Rybak LP, Whitworth CA, Mukherjea D, Ramkumar V. 2007. Mechanisms of cisplatin-induced ototoxicity and prevention. Hear Res. 226:157-167

[6] Gutherie OW. 2008. Aminoglycoside induced ototoxicity. Toxicology. 2008 Jul 30;249(2-3):91-6

[7] Haydel SE. 2010. Extensively drug-resistant tuberculosis: A sign of the times and an impetus for antimicrobial discovery. Pharmaceuticals 3:2268–2290

[8] Chen Y, Huang WG, Zha DJ et al. 2007. Aspirin attenuates gentamicin ototoxicity: from the laboratory to the clinic. Hearing research 226:178-182

[9] Dulon D, Aran JM, Zajic G et al. 1986. Comparative uptake of gentamicin, netilmicin, and amikacin in the guinea pig cochlea and vestibule. Antimicrobial agents and chemotherapy. 30:96-100

[10] Henley CM, Schacht J. 1988. Pharmacokinetics of aminoglycoside antibiotics in blood, inner-ear fluids and tissues and their relationship to ototoxicity. Audiology 27:137-146

[11] Steyger PS, Karasawa T . 2008. Intra-cochlear trafficking of aminoglycosides . Commun Integr Biol.1:140-2.

[12] Roland PS, Rutka JA. 2004. Ototoxicity. Hamilton (ON): BC Decker Inc, pp 85

[13] Walker EM Jr, Fazekas-May MA, Bowen WR . 1990. Nephrotoxic and ototoxic agents. Clin Lab Med. 10:323-54.

[14] Clerici WJ, Hensley K, DiMartino DL et al. 1996. Direct detection of ototoxicant-induced reactive oxygen species generation in cochlear explants. Hear Res. 98:116–124

[15] Sha SH, Schacht J. 1999. Formation of reactive oxygen species following bioactivation of gentamicin. Free Radic Biol Med. 26:341–347

[16] Hirose K, Hockenbery DM, Rubel EW. 1997. Reactive oxygen species in chick hair cells after gentamicin exposure in vitro. Hear Res. 104:1–14

[17] Sha SH, Qiu JH, Schacht J. 2006. Aspirin attenuates gentamicin-induced hearing loss. New Engl J Med. 354:1856–1857

[18] Forge A, Schacht J. 2000. Aminoglycoside antibiotics. Audiol Neurootol 5: 3-22

[19] Agrawal Y, Platz EA, Niparko JK. 2008. Prevalence of hearing loss and differences by demographic characteristics among US adults: data from the National Health and Nutrition Examination Survey, 1999-2004. Arch Intern Med. 168:1522-30

[20] Hallam R. Ashton P. Sherbourne K. Gailey L. 2006. Acquired profound hearing loss: mental health and other characteristics of a large sample. Int J Audiol. 45:715–723

[21] Reiter, RJ, Tan DX, Korkmax A et al. 2011. Drug-mediated ototoxicity and tinnitus: alleviation with melatonin." Journal of Physiology and Pharmacology 62: 151

[22] Groves AK. 2010. The challenge of hair cell regeneration. Exp Biol Med (Maywood). 235:434-46

[23] Mizutari K, Fujioka M, Hosoya M et al. 2013. Notch Inhibition Induces Cochlear Hair Cell Regeneration and Recovery of Hearing after Acoustic Trauma. Neuron 77: 58-69

[24] Stone J, Puel JL . 2013. Future cures for hearing impaired.[online] Available from: http://www.cochlea.org/en/future-cures. Accessed 9/2/13

[25] Bouillon, J., C. Gravili, F. Pages et al. 2006. An Introduction to Hydrozoa. Paris, France: Publications Scientifiques du Muséum. Available from: DOI:10.1086/586983. Accessed 8/24/13

[26] Arai, M. N. 1997. A functional biology of Scyphozoa. London (UK): Chapman & Hall. pp 316

[27] Wrobel, D. 2009.The Jellies Zone - Jellyfish And Other Gelatinous Zooplankton. Available at: http://jellieszone.com/. Acccessed August 31st, 2013.

[28] Schuchert, P. 2009. The Hydrozoa Directory. Available at: http://www.ville-ge.ch/mhng/hydrozoa/hydrozoa-directory.htm. Accessed August 31st, 2013

[29] Lucas CH, Lawes S. 1998. Sexual reproduction of the scyphomedusa Aurelia aurita in relation to temperature and variable food supply. Mar. Biol. 131: 629–638

[30] Lucas CH. 2001. Reproduction and life history strategies of the common jellyfish, Aurelia aurita, in relation to its ambient environment. Hydrobiologia 451: 229-246

[31] Spangenberg, DB. 1965. Cultivation of the life stages of Aurelia aurita under controlled conditions. J. exp. Zool. 159: 303–318

[32] Spangenberg DB. 1968. Recent studies of strobilation in jellyfish. Oceanogr. Mar. Biol. Ann. Rev. 6: 231–247

[33] Miyake H, Iwao K, Kakinuma Y. 1997. Life history and environment of Aurelia aurita. S. Pacific Stud. 17: 273–285

[34] Garm A, Ekstrom P, Boudes M et al. 2006. Rhopalia are integrated parts of the central nervous system in box jellyfish. Cell and Tissue Research 325: 333-343

[35] Martin, V.J., 2002. Photoreceptors of cnidarians. Can. J. Zool. 80, 1703–1722

[36] Brusca GJ, Brusca RC. 2003. Invertebrates, 2nd Edition. Sunderland: Sinauer Associates, Inc.

[37] Galliot B. 2010. Neurogenesis: Cellular and Molecular Cues from Cnidarian Nervous Systems. In: Key Transitions in Animal Evolution, eds Schierwater B, De Salle R. New York (NY): Science Publishers & CRC Press, pp. 127-161

[38] Albert DJ. 2008. Adaptive behaviours of the jellyfish Aurelia labiata in Roscoe Bay on the west coast of Canada. J. Sea Res. 59, 198–201

[39] Albert DH. 2011. What's on the mind of a jellyfish? A review of behavioral observations on Aurelia sp. jellyfish. Neuroscience and Biobehavioral Reviews 35: 474-482

[40] Arkett SA, Mackie GO. 1988. Hair Cell mechanoreception in the jelly fish Aglantha Digitalis. J. Exp. Biol. 135: 329-342

[41] Singla CL, Weber C.1982. Fine structure studies of the ocelli of Polyorchis Penicillatus (Hydrozoa), Anthomedusae) and their connection with the nerve ring. Zoomorphology 99, 117–129

[42] Flock A, Wersall J. 1962. A study of the orientation of the sensory hairs of the receptor cells in the lateral line organ of fish, with special reference to the function of the receptors. J. Cell Biol. 15, 19-27

[43] Shotwell SL, Jacobs R, Hudspeth AJ. 1981. Directional sensitivity of individual vertebrate hair cells to controlled deflection of their hair bundles. Ann. N.Y. Acad. Sci. 374: 1-10

[44] Hudspeth AJ, Jacobs R. 1979. Stereocilia mediate transduction in vertebrate hair cells. Proc. Natl. Acad. Sci. U.S.A. 76, 1506-1509

[45] Stone JS, Rubel. 2000. Cellular studies of auditory hair cell regeneration in birds. EW Proc Natl Acad Sci U S A.24; 97: 11714–11721

[46] Cruz RM, Lambert PR, Rubel EW. 1987. Light microscopic evidence of hair cell regeneration after gentamicin toxicity in chick cochlea. Arch Otolaryngol Head Neck Surg. 113:1058-62

[47] Wu WJ, Sha SH, McLaren JD et al. 2001. Aminoglycoside ototoxicity in adult CBA, C57BL and BALB mice and the Sprague-Dawley rat. Hear Res.158(1-2):165-78

[48] Roberson DW, Rubel EW. 1994. Cell division in the gerbil cochlea after acoustic trauma. Am J Otol. 15:28-34

[49] Ruben RJ. 1969. The synthesis of DNA and RNA in the developing inner ear. Laryngoscope 79:546-56

[50] Staecker H, Praetorius M, Baker K et al. 2007. Vestibular hair cell regeneration and restoration of balance function induced by math1 gene transfer. Otol Neurotol.28:223-31

[51] White PM, Doetzlhofer A, Lee YS et al. 2006. Mammalian cochlear supporting cells can divide and trans-differentiate into hair cells. Nature. 441:984–987

[52] Harris JA, Cheng AG, Cunningham LL et al. 2003.Neomycin-induced hair cell death and rapid regeneration in the lateral line of zebrafish (Danio rerio). J Assoc Res Otolaryngol.4:219-34

[53] Spangenberg, DB. 1994. Graviceptor development in jellyfish ephyrae in space and on earth. Advances in Space Research 14: 317-325

[54] Spangenberg DB, Jernigan T, Philput C. 1994. Graviceptor development in jellyfish ephyrae in space and on Earth. Adv Space Res. 1994;14(8):317-25

[55] Gershwin L. 2013. Stung!: On Jellyfish Blooms and the Future of the Ocean. University of Chicago Press, Page 91

[56] Ishii H, Kojima S, Tanaka Y. 2004. Survivorship and production of Aurelia aurita ephyrae in the innermost part of Tokyo Bay, Japan Plankton Biol. Ecol. 51: 26-35. 2004

[57] Zeddies DG, Fay RR. 2005. Development of the acoustically evoked behavioral response in zebrafish to pure tones. J Exp Biol 208: 1363-1372

[58] Fleming A. 2007. Zebrafish as an alternative model organism for disease modelling and drug discovery: implications for the 3Rs. NC3Rs #10. Available at: http://www.nc3rs.org.uk/news.asp?id=421. Accessed on: 8/27/13.

[59] Bianco IH, Ma LH, Schoppik D et al. 2012. . The tangential nucleus controls a gravito-inertial vestibulo-ocular reflex. Curr Biol. 22:1285-95

[60] Fleisch VC, Neuhauss SC. 2006. Visual behavior in zebrafish. Zebrafish 3: 191-201

[61] Dabiri JO, Colin SP, Costello JH. 2006. Fast-swimming hydromedusae exploit velar kinematics to form an optimal vortex wake. The Journal of Experimental Biology 209, 2025-2033

[62] McAfee S, Strasnick B. 2011. Jellyfish model for aminoglycoside toxicity. Otolaryngol Head Neck Surg 145 suppl 2: P96

[63] Hundgen M, Biela C. 1982. Fine structure of touch plates in the Scphomedusan Aurelia aurita. Q.J. Microsc Sci 97:59-74

[64] Budelmann B. 1988. Morphological diversity of equilibrium receptor systems in aquatic vertebrates. In: Sensory Biology of Aquatic Animals, ed. Attema J, Springer-Verlag, New York , 757-782

[65] Sprangenberg DB. 1991. Rhopalium development in Aurelia aurita ephyrae. Hydrobiologia 216/217: 45-49

[66] Pleis JR, Lethbridge-Cejku M. 2007. Summary health statistics for U.S. adults: National Health Interview Survey, 2006. National Center for Health Statistics. Vital Health Stat 10(235)

[67] Larson VD, Williams DW, Henderson WC. 2000. Efficacy of 3 commonly used hearing aid circuits. JAMA 284:1806-1813

[68] Perde-Schrepler M, Maniu A, Cosgarea M . 2012. Current strategies for the protection, regeneration, and replacement of cochlear hair cells. J Otolaryngol Head Neck Surg;41:227-39

[69] Arts HA. 2010. Sensorineural hearing loss in adults. In: Cummings CW, Flint PW, Haughey BH, et al, eds. Otolaryngology: Head & Neck Surgery. 5th ed. Philadelphia, Pa: Mosby Elsevier, chap 149

[70] Tabuchi K, Nishimura B, Nakamagoe M et al. 2011. Ototoxicity: mechanisms of cochlear impairment and its prevention. Curr Med Chem. 18:4866-71

[71]Selimoglu E. 2007. Aminoglycoside-induced ototoxicity. Curr Pharm Des. 13:119-26

[72] Best EJ, Gazarian M, Cohn R et al. 2011. Once-daily gentamicin in infants and children: a prospective cohort study evaluating safety and the role of therapeutic drug monitoring in minimizing toxicity. . Pediatr Infect Dis J.30:827-32

[73] Ahmed RM, Hannigan IP, MacDougall HG, Chan RC, Halmagyi GM. 2012. Gentamicin ototoxicity: a 23-year selected case series of 103 patients. Med J Aust. 196:701-4

[74] Black FO, Pesznecker S, Stallings V. 2004 Permanent gentamicin vestibulotoxicity. Otol Neurotol 25:559-69

[75] Janas JD, Cotanche DA, Rubel EW. 1995. Avian cochlear hair cell regeneration: stereological analyses of damage and recovery from a single high dose of gentamicin. Hear Res. 92(1-2):17-29