Transtympanic administration of dexamethasone: An innovative otoprotection against cisplatin chemotherapy

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TABLE OF CONTENTS

ABSTRACT II

RESUME III

ACKNOWLEDGEMENTS IV

PART ONE: INTRODUCTION, ANATOMY AND IMMUNOLOGY 1

CHAPTER 1. STUDY INTRODUCTION 1

CHAPTER 2. ANATOMY AND FUNCTION OF THE MAMMALIAN EAR 3

CHAPTER 3. IMMUNOPATHOLOGY OF THE INNER EAR 12

PART TWO: HEARING LOSS, OTOTOXICITY AND AUDIOLOGIC MONITORING 19

CHAPTER 4. HEARING LOSS 19

CHAPTER 5. OTOTOXICITY 23

CHAPTER 6. AUDIOLOGIC MONITORING FOR OTOTOXICITY 28

PART THREE: CISPLATIN OTOTOXICITY AND OTOPROTECTION 33

CHAPTER 7. CISPLATIN OTOTOXICITY 33

CHAPTER 8. OTOPROTECTION 39

CHAPTER 9. GLUCOCORTICOIDS 44

CHAPTER 10. TRANSTYMPANIC THERAPY 49

CHAPTER 11. DEXAMETHASONE AND CISPLATIN OTOTOXICITY 54

PART FOUR: ANIMAL STUDY 56

CHAPTER 13. EXPERIMENTAL DESIGN 56

CHAPTER 14. EXPERIMENTAL SET-UP 59

CHAPTER 15. RESULTS 61

CHAPTER 16. DISCUSSION 65

CHAPTER 17. CONCLUSION AND SUMMARY 70

REFERENCES 71

Abstract

Introduction: Cisplatin chemotherapy causes ototoxicity manifested as sensorineural hearing loss and/or tinnitus. Ototoxicity is induced via damage to the inner ear by reactive oxygen species. Dexamethasone reduces reactive species and has a well-documented history of transtympanic clinical use for various cochlear disorders.
Objectives: Determine the effect of transtympanic dexamethasone on cisplatin ototoxicity in a guinea pig animal model.
Material and Methods: Pre- and post-treatment Auditory Brainstem Responses (ABRs) were compared to measure threshold changes in 58 guinea pigs. The optimal ototoxic dose of cisplatin, the safety of dexamethasone and the effect of dexamethasone on cisplatin ototoxicity were examined.
Results: Cisplatin at a dose of 12 mg/kg induces significant hearing loss ($p < 0.05$) with minimal mortality. Transtympanic dexamethasone in cisplatin-treated guinea pigs showed signs of otoprotection particularly in the lower frequencies.
Conclusion: Transtympanic dexamethasone presents as a simple, safe and potentially effective treatment modality against cisplatin ototoxicity.

Résumé

Introduction: La chimiotherapie au cisplatin cause une perte auditive ototoxique induite par dommages à l'oreille interne par des espèces réactives. La dexaméthasone réduit les espèces réactives et a déjà fait ses preuves dans des traitements trans-tympaniques (TT) de pathologies cochléaires.

Objectifs: Déterminer l'effet de la dexaméthasone TT sur l'ototoxicité du cisplatin chez le cobaye.

Matériel et méthodes: Les potentiels auditifs évoqués du tronc cérébral (PTE) ont été comparés chez 58 cobayes. La dose ototoxique optimale du cisplatin, la sécurité de la dexaméthasone et l'effet de la dexaméthasone sur l'ototoxicité du cisplatin ont été examinés.

Résultats: 12 mg/kg de cisplatin induit une perte auditive significative ($p < 0.05$) avec une mortalité minime. La dexaméthasone TT a montré des signes d'otoprotection chez les cobayes traités au cisplatin surtout dans les basses fréquences.

Conclusion: La dexaméthasone TT se présente comme une modalité de traitement simple, sécuritaire et potentiellement efficace contre l'ototoxicité produite par le cisplatin.

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PART ONE: Introduction, Anatomy and Immunology

Chapter 1. Study Introduction

Since its discovery some 40 years ago, cisplatin has become a common chemotherapeutic agent used to treat a wide variety of cancer in adults and in children. Unfortunately, along with its antineoplastic effects, it has several toxic side-effects. The most common of these toxicities is ototoxicity. Cisplatin ototoxicity usually manifests as sensorineural hearing loss with or without tinnitus which begins in the high frequencies and progresses into the lower frequencies important for speech perception [1-2]. Ototoxicity is known to be cumulative, dose-related, bilateral and irreversible [1-2]. Up to a third of cisplatin-treated patients suffer from significant hearing handicaps, while up to 80% can show elevations in their hearing thresholds [3-4]. Cisplatin exerts this damage in the inner ear, resulting in the loss of the cochlear outer hair cells (OHCs), as well as cells of the stria vascularis and the spiral ganglion, ultimately leading to hearing loss.

Cisplatin is believed to exert its ototoxic affects through the actions of reactive species or free radicals [5]. Reactive species deplete the natural antioxidant system in the inner ear, resulting in cell death. There have been many attempts to inhibit the production and formation of these free radicals by the administration of antioxidants at various stages in the ototoxic pathways. Unfortunately, the majority of these agents, especially when given systemically, have been found to inhibit the antineoplastic effects of cisplatin, or exhibit toxicities of their own [6].

Presently, there is no treatment for cisplatin ototoxicity [5]. When ototoxicity develops, treatment of cancer with cisplatin is stopped and the use of a less potent antineoplastic agent (such as carboplatin) is used, or the amount of hearing loss is tolerated as an acceptable side-effect of chemotherapy. An effective treatment for cisplatin ototoxicity must protect the ear from hearing loss without interfering with the chemotherapeutic effects of cisplatin.

The glucocorticoids, such as prednisone, methylprednisolone, and dexamethasone, are a class of drugs that have potential for otoprotection. Many different cochlear disorders, including Ménière's disease, tinnitus, and autoimmune inner ear diseases are currently treated with systemic, as well as transtympanic glucocorticoids [7-8]. Powerful anti-inflammatories, glucocorticoids have been shown to limit the production of reactive species and inflammatory molecules in the inner ear [9-11].

Intratympanic (IT), or transtympanic, administration of drugs is an effective method for locally delivering treatment to the inner ear. In fact, IT administration of steroids has been clinically used to treat conditions such as Ménière's disease for many years [7]. IT administration allows for the direct application of treatment into the middle ear, allowing it to diffuse across the round window and into the inner ear where it can exert its effects. Diffusion of steroids across the round window into the inner ear to bathe the inner ear structures has been well documented. Local application allows for the concentration of a much higher level of steroid in the inner ear compared to systemic routes. IT administration also avoids any interference with the efficacy of chemotherapy, as well as common systemic side affects of steroids, like ulcers, hypertension, or osteoporosis.

The present study was designed to test the effect of dexamethasone on cisplatin ototoxicity. Experiments were carried out in a well-established guinea pig model. The objectives were to determine the optimal ototoxic dose of cisplatin, to determine the safety of dexamethasone, and to investigate its protective effects against cisplatin ototoxicity.

Chapter 2. Anatomy and Function of the Mammalian Ear

Andreas Vesalius (1514-1564), paved the way for a shift in understanding human anatomy. Vesalius, along with other Italian anatomists Gabriele Fallopio, Bartolomeo Eustachio, and Giovanni Ingrassia, investigated the inner ear and are credited with many of the first accurate descriptions of its structures [12]. Since that time, many different discoveries of ear anatomy and physiology have lead to modern day understandings and significant advances in the pathophysiology of otologic conditions, surgical approaches and administration of medications.

The ear is the organ of hearing and equilibrium. It transforms sonic vibrations into nervous impulses that are interpreted by the brain as sound. The ear is also responsible for maintaining balance and equilibrium through nerve endings located in the vestibular apparatus. All mammals have two ears consisting of three divisions: the external, the middle and the inner, or internal, ear. The main structures of the ear are all encased in the petrous temporal bones of the skull. The aim of this study is directed towards the prevention of hearing loss; thus, the following anatomical descriptions will focus on the main structures and cells of the auditory pathway.

The External Ear and Tympanic Membrane

The external ear has a funnel-shaped outer part called the auricular or pinna and is composed of skin and elastic cartilage. From the pinna, the external ear continues into the skull by a membrane-lined tube, the external acoustic meatus, or ear canal.

The eardrum, or tympanic membrane, separates the external ear from the middle ear. It is the only tissue that is derived from all three of the original germ layers of the embryo (the ectoderm, mesoderm and endoderm) [13]. It functions as a vibratory structure, allowing sound waves to be converted into mechanical energy.

The Middle Ear

The middle ear is a hollow chamber encased by temporal bone. It contains the ear bones, or ossicles, the malleus, the incus and the stapes. The middle ear has four openings: the external acoustic meatus, the *fenestra ovalis* or oval window, the *fenestra rotundum* or round window, and the Eustachian tube. The Eustachian tube is the only opening free of membrane partitions. It connects with openings in the walls of the nasopharynx and functions to equalize the pressure between the middle and external ear.

The ossicles are connected to each other and to the walls of the cavity of the middle ear by delicate ligaments. The malleus is also attached to the inner surface of the tympanic membrane, and the stapes is attached to the outer surface of the oval window membrane. The ossicles vibrate in response to the movement of the tympanic membrane, and function to transmit and amplify energy to the inner ear. The round and oval windows separate the middle ear from the inner ear.

The Inner Ear

The internal ear is essential to hearing and equilibrium in mammals. Because of its complex shape it is termed the labyrinth and can be divided into two parts; the osseous labyrinth and the membranous labyrinth. In humans, the inner ear takes up a volume of approximately 200 μL [14-16]. The guinea pig inner ear is approximately 10 times smaller, taking up a volume about 20 uL [17].

The osseous labyrinth contains the vestibule, the semicircular canals and the cochlea. It is filled with a watery fluid called perilymph and surrounds soft tissue of the membranous labyrinth. The vestibule is the central part of the osseous labyrinth, communicating with the cochlea in front and the semicircular canals behind. The external wall contains the oval window. The cochlea resembles a snail's shell, the origin of its name. It is a conical and spiralled structure located in the rostral part of the labyrinth. It contains the organ of hearing, the organ of Corti.

The membranous labyrinth is a series of closed membranous sacs containing endolymph. The vestibule contains two membranous sacs, the utricle and saccule. The saccule is united with the cochlear duct, which is a spiral tube winding the length of the cochlea. This duct contains the organ of Corti. The utricle and saccule are both connected to the endolymphatic duct, which proceeds to the dura mater of the brain along with the auditory nerve. Here, the endolymphatic duct expands to form the endolymphatic sac [13]. In guinea pigs, the endolymphatic sac takes up a volume from 0.1 to 0.17 μL [17-18].

The cochlea

The cochlea consists of a single bony tube, the cochlear duct, which spirals around a middle 'core', or modiolus that contains the spiral ganglion of the auditory nerve. In humans, the coiled duct makes about two and two-thirds turns with a total length of approximately 34 mm [19]. In guinea pigs, the cochlea is shorter and more coiled with approximately three and a half turns and an overall length of about 19 mm (Figure 1) [20].

The cochlear duct is divided by membranous tissue into three separate chambers called scalae: the scala vestibule, the scala media, and the scala tympani (Figure 2). These two compartments are connected, and communicate at the apex of the cochlea by a small hole called the helicotrema. The oval window touches the scala vestibuli and the round window touches the scala tympani. In otology, the round window is important for drug diffusion into the cochlea (Ch.10). Humans have round window membranes of an average thickness of approximately 69 μm, while guinea pig round window membranes have been measured at 10 to 30 μm thick [21-22].

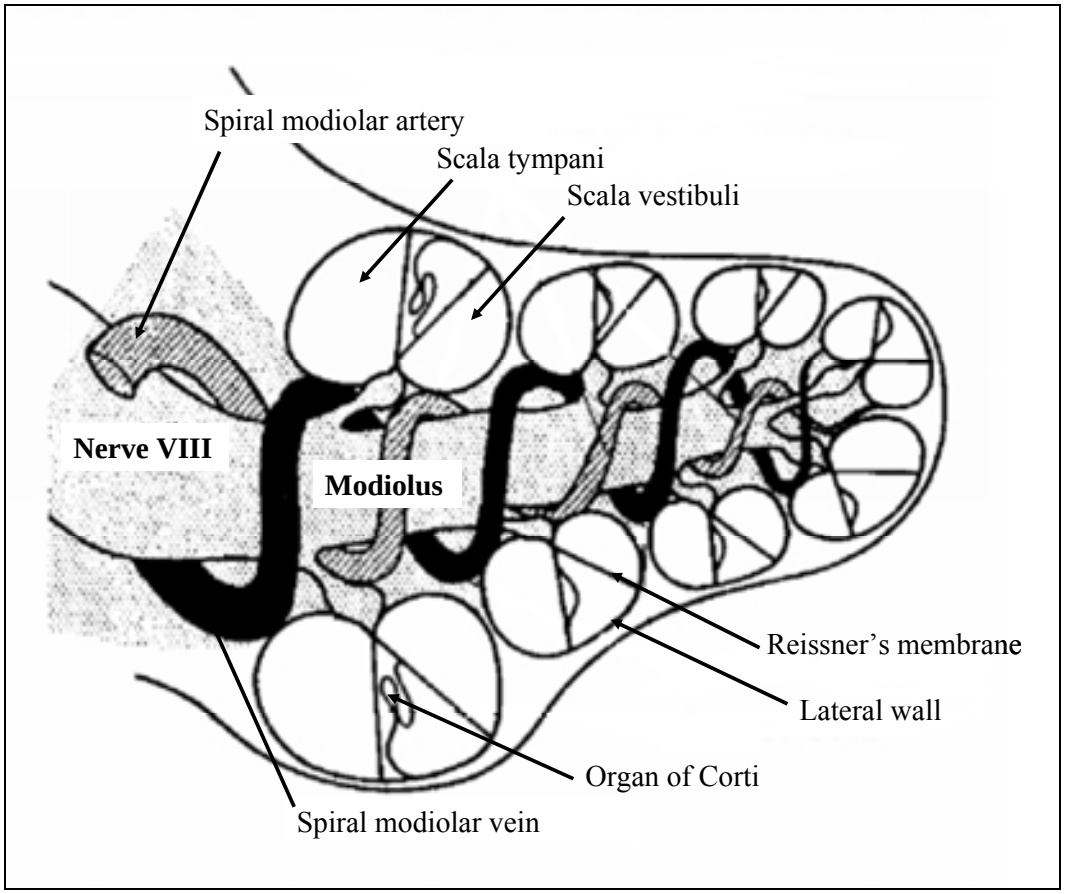

Figure 1. Schematic drawing of the guinea pig cochlea displaying various anatomical structures. Modified from Jiang, Oregon Hearing and Research Center, Oregon Health & Science University [23].

As its name suggests, the scala media lies in between the scala vestibuli and the scala tympani. In humans, each scala measures approximately 30 mm with volumes measured from 29 to 44 uL [15, 24]. In guinea pigs, each scala measures 15 to 16 mm long with a volume of roughly 4 to 5 μL [17, 24]. The scala tympani and the scala vestibule both contain perilymph and the scala media contains endolymph. Endolymph has a high concentration of potassium (K^+) with almost no sodium, while perilymph has a high concentration of sodium (Na^+) [19]. The endolymph-filled scala media is bound by the lateral wall (stria vascularis), Reissner's membrane and the basilar membrane. Reissner's membrane separates the scala media from the scala vestibuli, while the basilar membrane separates the scala media from the scala tympani.

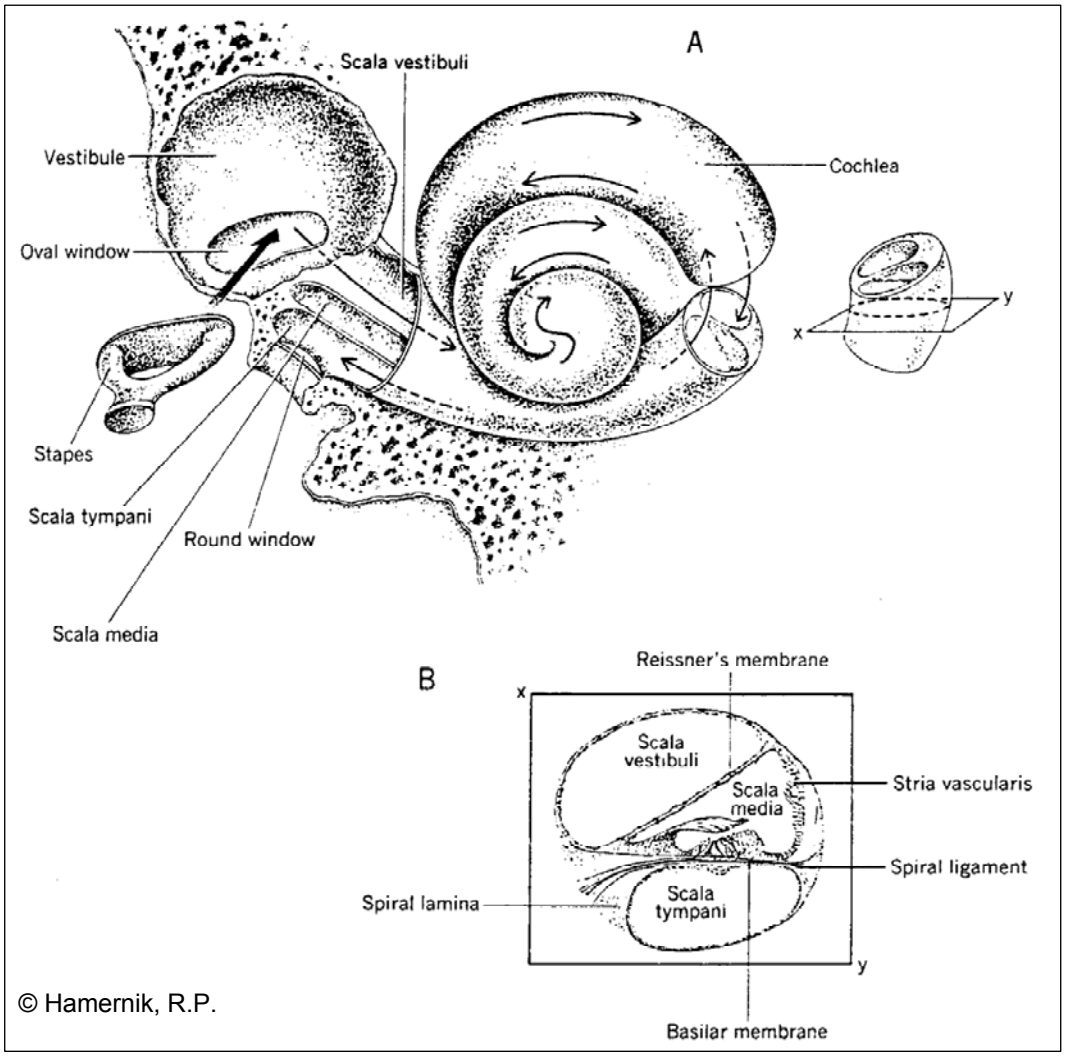

Figure 2. From Hamernik *et al.* [25-26]: A. The cochlea of the right ear displaying the relative positions of the scala vestibule, scala media and scala tympani with arrows displaying possible sound pathways. B. Cross section of the cochlear tube.

The lateral wall is composed of the spiral ligament and the stria vascularis and it acts to maintain the high K^+ concentration in the endolymph. The spiral ligament contains two different types of tissue required for ion transport activity: type I fibrocytes adjacent to the stria vascularis, and type II fibrocytes near the basilar membrane. Type I fibrocytes recycle K^+ ions from endolymph to the stria. Other fibrocytes (predominantly type I) contain stress fibres to supply tension to the basilar membrane. The three main cell types of the stria vascularis are marginal, intermediate and basal cells. The marginal cells contain a high amount of Na^+/K^+ adenosine triphosphase (ATPase) and many mitochondria, all required for

pumping K^+ into the endolymph. The basal cells communicate with the intermediate cells via gap junctions and are in direct communication with cells of the spiral ligament. The intermediate cells are located closest to the vasculature and are essential in ion flow for generating an endocochlear potential.

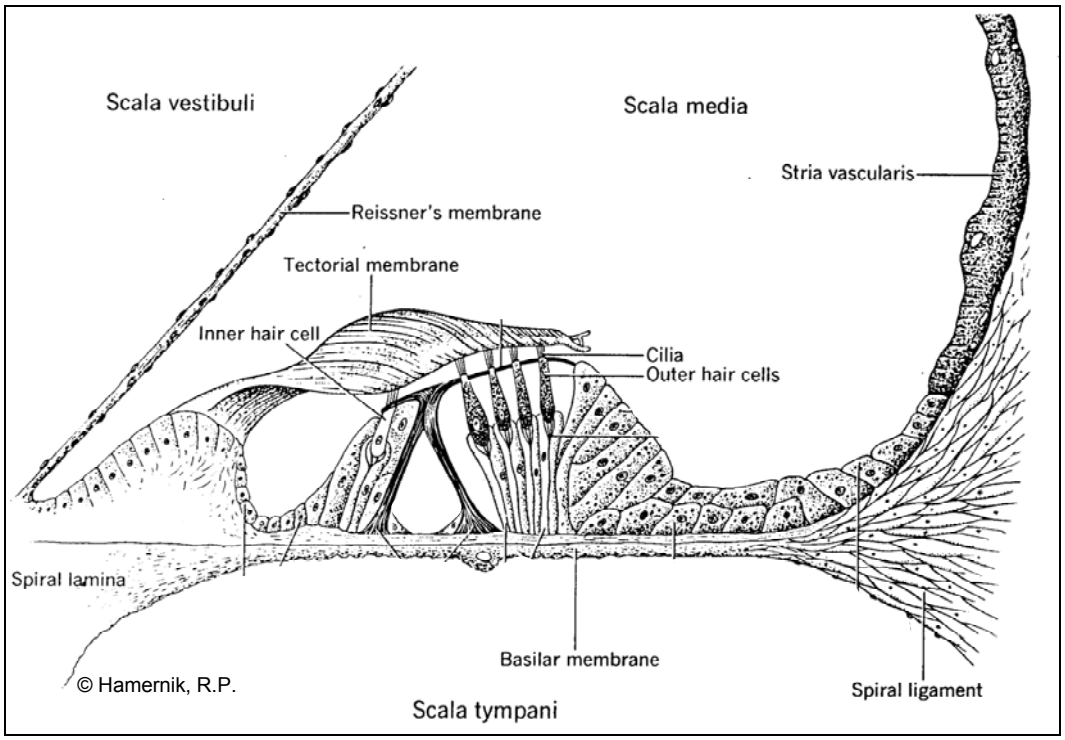

Figure 3. Cross section of the scala media showing the organ of Corti, including the inner and outer hair cells, modified from Hamernik [25-26].

The organ of Corti is the sensory epithelium of the cochlea (Figure 3). It lies all along the basilar membrane and is formed by rod-shaped, supporting cells, hair cells and the tectorial membrane. The tectorial membrane extends above the hair cells from the inner side of the organ of Corti.

The hair cells are the sensory cells responsible for transducing mechanical stimuli into electrical signals. They derive their name from the tufts of stereocilia (a hair bundle) that protrude from their apical surfaces into the scala media. Mammalian cochlear hair cells come in two anatomically and functionally distinct types: inner hair cells and outer hair cells. In human ears, there are approximately 16 000 to 20 000 hair cells along the length of the cochlea. There is a single row of inner hair

cells and three rows of outer hair cells, extending from the base to the apex of the cochlea [19]. Both hair cells have hair bundles of 50 to 150 stereocilia in a long-to-short gradient arrangement [19]. Only stereocilia of the outer hair cells are in direct contact with the tectorial membrane.

The spiral ganglion is contained within the modiolus. The bipolar sensory neurons of the spiral ganglion extend into the basilar membrane and synapse on the basal side of the inner and outer hair cells. There are two types of bipolar sensory neurons in the spiral ganglion. The majority (90 to 95%) are type I cells whose fibres contact inner hair cells [19]. Type II cells synapse with outer hair cells. The neuronal processes of type I and II cells form the cochlear branch of the vestibulocochlear nerve. Olivocochlear efferent nerve fibres run along the basilar membrane to contact the inner and outer hair cells.

Tonotopy

From the Greek word meaning 'the place of tones', tonotopy is the spatial arrangement of where sound is perceived, transmitted, or received. The sensory epithelium of the cochlea is tonotopically arranged, as is the part of the brain that processes sound information, the auditory cortex [27]. High frequencies are detected from stimulation at the base of the cochlea which progress up to low frequency sound-detection near the apex of the cochlea [28].

The Process of Hearing

There are two factors that play an essential role during the hearing process: ion concentrations and fluid movement. The high concentration of K^+ in the endolymph and the high concentration of Na^+ in the perilymph result in an electrical potential difference. The endocochlear potential is essential for sensorineural transduction. This ion concentration is regulated by the secretory and absorptive activity of the stria vascularis. Fluid movement in the scala tympani (caused by sound vibrations carried from the tympanic membrane through the ossicles) induces movement of the basilar membrane. Movement of

the basilar membrane causes the taller of the stereocilia to be displaced by the tectorial membrane. This deflection results in opening of ion channels at the tip of the stereocilia, driving K^+ into the cell which becomes depolarized. Upon depolarization, an influx of Calcium (Ca^+) causes the release of neurotransmitter at the hair cell-cochlear nerve fibre synapse and generates a stimulus, interpreted by the auditory cortex in the brain [19].

Neurons of the auditory or vestibulocochlear nerve (VIIIth cranial nerve) innervate the cochlear hair cells. Glutamate is thought to be the neurotransmitter released by the hair cells to stimulate dendrites of afferent neurons. At the presynaptic juncture there is a presynaptic dense body, called a ribbon. The ribbon is surrounded by synaptic vesicles and is thought to aid in the fast release of neurotransmitter. The inner hair cells are connected to approximately 90% of auditory innervations [19]. Thus, the inner hair cells are responsible for the majority of neural signals interpreted as sound. The outer hair cells, with the minority of innervations (5-10%) act to modify, and regulate incoming signals [19]. Inner hair cell nerve fibres are also heavily myelinated, in contrast to the unmyelinated outer hair cell nerve fibres. There are also efferent synapses on outer hair cells and on afferent dendrites under the inner hair cells. Acetylcholine and the neuropeptide Calcitonin gene related peptide (CGRP) are the neurotransmitters here. The effects of these compounds vary, in most cases acting as in part as feedback to locally reduce the sensitivity of the cochlea.

The Importance of Hair Cells

The inner ear is an active organ. In addition to its main function of 'receiving' sound, the inner ear may also generate sound (see OAEs, Ch. 6). This sound generation occurs when outer hair cells alter their cell bodies enough to move the basilar membrane to produce or pre-amplify sound. This process is important in fine-tuning the ability of the cochlea to accurately detect differences in incoming acoustic stimuli. Whereas most bird species do not hear above 5 kHz, some marine mammals can hear up to 200 kHz [29]. The superior frequency resolution in

mammals is due to this mechanism of pre-amplification of sound by active cell-body vibration of the outer hair cells [29]. In addition, the large, coiled cochlea of mammals, unlike than the short straight cochlea of birds or other vertebrates, provides sufficient space for frequency dispersion and is therefore better adapted to the specific frequency resolution in mammalian hearing [29].

With approximately 17 000 hair cells, the human cochlea contains far fewer receptor cells than other sensory organs, like the retina or olfactory epithelium [30]. Due to this lack of significant redundancy, the cochlea is severely damaged by the loss of even a few thousand hair cells. Although birds and fish can regenerate new hair cells after deafening, humans have lost the ability to regenerate hair cells; once a human cochlear hair cell has died, it is gone for good [30]. Therefore, the loss of hair cells due to trauma or ototoxic drug damage is permanent (Ch.4, Ch. 5). Hair cell regeneration is currently being investigated as an eventual therapy for hearing loss. However, researchers have so far been unsuccessful in developing hair cell regeneration in humans [30]. For this reason, the protection of human cochlear hair cells remains an essential area of research for hearing loss prevention.

Chapter 3. Immunopathology of the Inner Ear

The immunity of the inner ear is similar to that of the brain [31]. It lacks lymphatic drainage and is separated from the serum by the blood-labyrinthine barrier which helps maintain the ionic balance of the fluids of the inner ear [32]. Very low concentrations of immunoglobulins are present in the perilymph. White blood cells enter the cochlea through the spiral modiolar vein and are responsible for the local production of immunoglobulins. In a healthy inner ear, lymphocytes are present in the endolymphatic sac, but absent in the cochlea.

The immunopathology of the inner ear involves a multitude of complex, radiating and multifunctional pathways, cells, signalling molecules and enzymes. To better understand the mechanisms of cellular damage and immunopathology in the inner ear, significant signalling molecules, cellular proteins and intermediate enzymes will be outlined.

Cytokines

Cytokines are a category of signalling molecules that are used extensively in cellular communication. They are proteins and peptides secreted by cells that carry signals between cells, surface receptors and other functional enzymes. They encompass a large, diverse family of regulators that are produced widely throughout cells of the body. Specifically, interleukins and tumour necrosis factors are cytokines that play a major role in the immune response of the inner ear.

Interleukins (ILs) are a group of cytokines that were first expressed by leukocytes (white blood cells), but are known to be secreted by many different cell types. The proper function of the immune system is especially dependent on this group of cytokines. There are over 30 different kinds of ILs, each with a variety of wide-reaching functions. Interleukin-1 (IL-1), interleukin-2 (IL-2), and interleukin-6 (IL-6) are all of particular importance for immune responses in the inner ear.

IL-1 and IL-2 both have similar signalling responsibilities during cell damage in the inner ear. Together, they target T-cells, B-cells, natural killer (NK) cells, macrophages, and many other non-immunocompetent cells of the body. They are involved in the stimulation, activation, maturation and proliferation of these cells and their proteins. IL-2 has specific functioning in the growth and differentiation of these cells. These ILs are also both directly and indirectly responsible for inflammation in the inner ear. IL-6 has similar target cells, but has a particular role in the secretion of antibody. Like IL-1 and IL-2, IL-6 is directly implicated in causing inflammation [33-34].

The Tumour Necrosis Factor (TNF) family is another group of cytokines that are mainly associated with cell death. There are two main types of TNFs in this family: TNFα and TNFβ. TNFβ has a more minor and specific role in systemic immunity. TNFα is the most well-known of the family, and has a major role in the immune response in the inner ear. The TNFs act through TNF receptors (TNFR). They are part of the extrinsic pathway for triggering cell death most often through apoptosis and also cytolysis. TNFα is released in response to IL-1 and can work together with IL-1 and IL-6 in activating and increasing the immune response. It plays a main role in inflammation, inducing an inflammatory response and regulating immune cells. It can interact with endothelial cells leading to increased vascular permeability and infiltration of immune cells to the site of damage. It also increases the activation of phagocytosis and the production of the inflammatory lipid prostaglandin which is directly involved in inflammation.

Cellular Proteins

Nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) and the mitogen activated protein kinase (MAPK) family of proteins are two cellular proteins involved in an extremely wide variety of cellular activities including immunopathology and inflammatory processes [35-36]. These proteins are found on or within almost every cell of the body and are key in regulating cell function and death.

NFkB is a protein complex particularly controlling the transcription of DNA. It is extremely ubiquitous in distribution, found in almost all animal cell types. This protein complex is directly involved in the cell's responses to many different stimuli, including stress, cytokines, free radicals, ultraviolet radiation, and foreign antigens. NFkB also plays a key role in the regulation of inflammation and the immune response. Incorrect functioning of this factor has been linked to cancer, inflammatory and autoimmune conditions, and septic shock and infection. NFkB is a rapid-acting, primary transcription factor; it does not require new protein synthesis to become activated. It is stimulated by ILs and TNFs from immune cells and other cells of the body. For example, binding of TNFα to the TNFR can lead directly to NFkB activation and rapid changes in gene expression. Foreign antigens can also directly activate NFkB. This factor is responsible for important cellular functions including control of cell proliferation and survival, cellular immune responses and cell death.

Mitogen-activated protein kinases (MAPKs) are a family of cellular enzymes that also respond to extracellular stimuli. Their role in inner ear immunopathology is similar, but much less multifaceted as that of NFkB. They are involved in the regulation of cellular activities such as some gene expression, mitosis, differentiation and proliferation and ultimately, cell survival. However, their main activity in the inner ear immune response is their ability to initiate apoptotic pathways.

Interaction of Cytokines and Cellular Proteins

Cell death in the inner ear during an immune response can be attributed to the combined effects of cytokines on cellular proteins. TNFα may interact with NFkB and the MAPK family to directly trigger apoptosis and/or apoptotic pathways. It may also interact with NFkB to initiate and mediate a vast array of proteins involved in cell survival, proliferation and the inflammatory response.

Intermediate Enzymes

Enzymes are mainly proteins that catalyze chemical reactions, such as the synthesis of molecules. In the immunopathology of the inner ear, the enzymes NADPH Oxidase (NOX) and Nitric Oxide Synthase (NOS) are of main importance. During immune responses in the inner ear, especially initiated by the mechanisms of cisplatin, these intermediate enzymes are responsible for the synthesis of molecules that may initiate and fuel the inflammatory process and directly lead to cellular damage and cell death.

NOX is a membrane-bound protein found in the plasma membrane or the membrane of the phagosome. It occurs in most cells, especially neutrophils, and has normal functions during cellular respiration and detriment processing. NOX, specifically NOX3, has been shown to exist in the inner ear at concentrations 50-fold higher than other tissues such as the kidney [37]. NOX generates superoxide by transferring electrons from NADPH. Superoxide is a toxic free radical, or part of the class of reactive oxygen species (ROS). It is usually produced safely within the cell during normal processes, but can also be produced outside of the cell during immune and inflammatory activation. Therefore, NOX is a critical enzyme in the initiation the inflammatory response and cellular damage and cell death, through the ROS pathway.

NOS is an enzyme that catalyzes the production of nitric oxide (NO). NO is an important molecule in the body and is produced safely in small quantities for important functions such as cell communication. There are three types of NOS: nNOS in nervous tissue, eNOS in endothelial tissue and, most importantly, iNOS which is involved in the immune response. nNOS and eNOS are both involved in cell communication and vasodilation in nervous and endothelial tissue, but have no strong roles in inner ear immunopathology. iNOS is the enzyme directly involved with the immune response and inflammation.

iNOS is directly and indirectly activated by IL-1 and IL-2, respectively. iNOS can also be synthesized in response to inner ear damage from ROS such as superoxide. iNOS acts to increase the amount of NO. As mentioned, small amounts of NO are not very toxic, but an overproduction of NO can be damaging to cells. More importantly, NO can react with superoxide (from NOX) to form peroxynitrite, an extremely reactive nitric oxygen species. In this way, iNOS and NOX are essential in the production of reactive oxygen and reactive nitrogen species which ultimately lead to cell damage, cell death and a potentiation of the inflammatory response.

The Natural Immune Response in the Inner Ear

Due mainly the blood-labyrinthine barrier, the inner ear was historically considered to be an immunopriviledged site in the body [32]. However, it is now known that the inner ear is capable of mounting a strong immune response to foreign antigens or to damage [31, 38].

The beginnings of a local immune response within the inner ear can be detected within hours of damage or invasion of a foreign antigen. The immune response is characterized first by the arrival of macrophages and neutrophils, followed by T cells and B cells [38]. If the offence to the inner ear persists an antibody-specific response may be generated within approximately 4 to 7 weeks [38].

At rest, the cochlea itself contains no immunocompetant cells [38]. The endolymphatic sac (ES) does contain immunocompetent cells and is the first site of the immune response for the inner ear. It is here that responses to assaults within the cochlea are mounted (Figure 4). This is evidenced by the observation that even with foreign antigen directed at specific locations within the cochlea only, antigen-presenting cells first appear within the ES [38]. Antigens have been shown to diffuse to the ES through the perilymph and perisaccular tissues, with the perilymph being resorbed from the inner ear by the spiral ligament (SL).

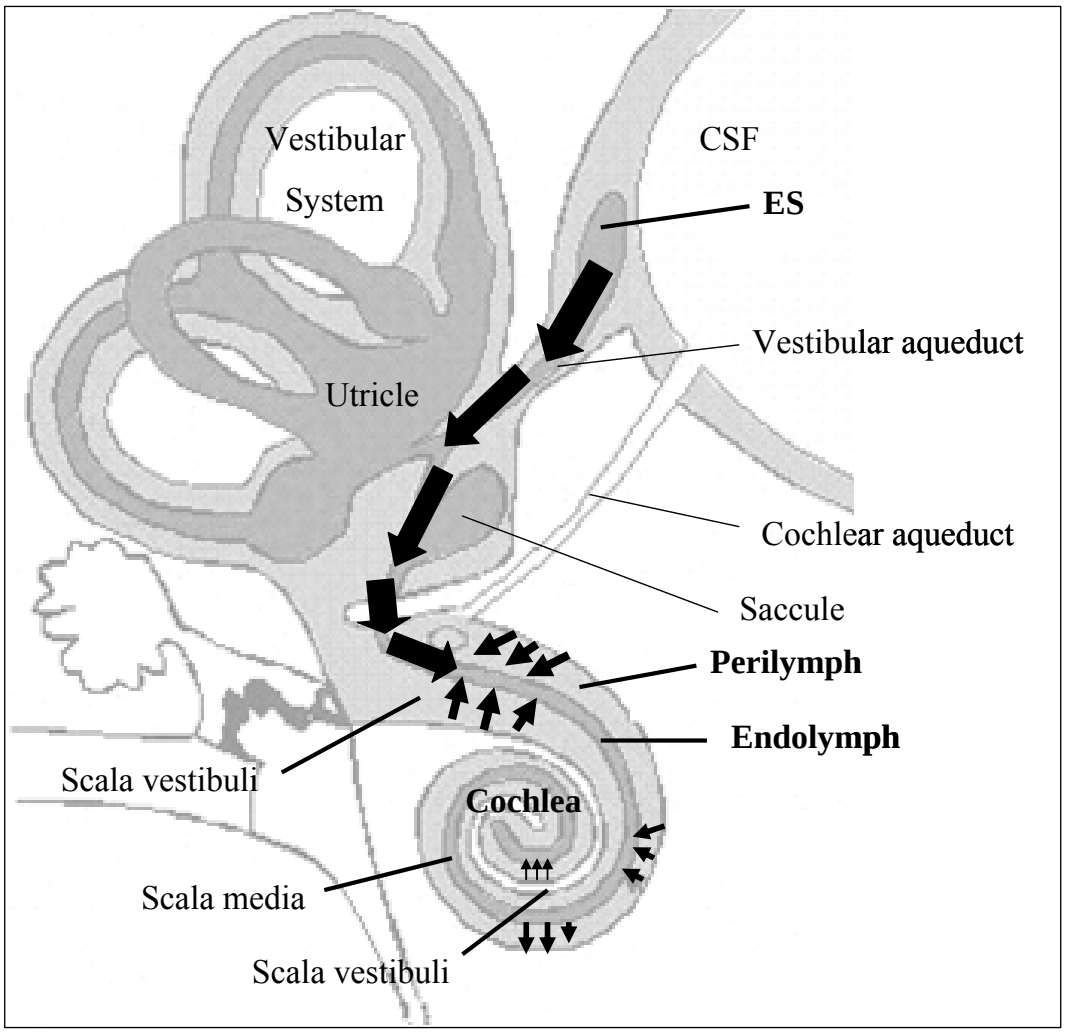

Figure 4. Diagrammatic representation of the generalized flow of immunocompetant cells and molecules during an immunological response in the cochlea. Large arrows represent the flow of immunocompetant cells from the endolymphatic sac (ES). Smaller arrows represent the perilymphatic and vascular infiltration of cells, adhesion molecules and cytokines directly in the cochlea. (McGill Auditory Sciences Laboratory, 2010; modified from diagram of Alec Salt, Washington University) [39].

At rest, perilymph of the inner ear has been shown to contain very small amounts of systemic antibody; about 1/1000th of that of found in serum [38]. During an immune reaction, systemic cells can also be found in the cochlea. These cells have been shown to infiltrate into the scala tympani and to a lesser extent, the scala vestibuli. Their infiltration appears to occur along the spiral modiolar vein (SMV). This vein has been shown to undergo changes in morphology in response to an immune reaction. These changes include larger nuclei and increased cytoplasm.

An increase in inflammatory cytokines, such as TNFα and adhesion molecules like ICAM also arrive in the SMV. They facilitate lymphocyte and other cell adherence to and infiltration through the vascular wall [38].

Along with the immune response in the inner ear is a steady increase in the extracellular matrix. Fibroblasts appear as the extracellular matrix increases (usually by the first day). It is important to note that fibroblasts themselves are important mediators of cochlear homeostasis and inflammatory pathways. They are capable of inducing inflammation directly through the secretion of signalling molecules such as TNFα or IL-1 [40-42]. Thus, fibrocytes may be responsible for the indirect induction of NOX, resulting in reactive oxygen species generation (See Ch. 7, Ch. 11).

Because of the increase in extracellular matrix, if the immune response persists, the inner ear is in danger of ossification. Indeed, fibrotic tissues have been shown to be present within one week, and ossification has been shown to begin by 3 weeks [38]. Ossification brought on by the inflammatory increase in extracellular matrix in a common problem seen in many conditions affecting the inner ear including labyrinthitis, otosclerosis and trauma.

Another interesting aspect of inner ear immunity is that cellular damage appears to be irrespective of the site of infection or initial trauma [38]. Damages resulting from the immune response are mostly all seen in the organ of Corti, the stria vascularis, and the spiral ganglion. Also, the amount of damage appears to be related to the magnitude of the immune response. A more robust immune response will incur greater damage to the inner ear. Unfortunately, the inner ear seems to be particularly sensitive to an immune response and generally ineffective at clearing the resulting inflammatory pathways [32].

PART TWO: Hearing Loss, Ototoxicity and Audiologic Monitoring

Chapter 4. Hearing Loss

A hearing loss, impairment, or deafness is a complete or partial inability to perceive at least some frequencies of sound within the normal range of hearing. For humans, this range is approximately 20 to 20 000 Hz. Generally defined, hearing loss is deterioration in hearing, with profound hearing loss resulting in deafness. Hearing loss becomes more common among older people (presbycusis) and has many causes. Most hearing loss is not treatable, except with hearing aids and in some cases surgery. Over 28 million people in the US are deaf or have hearing loss, with up to 40% of people aged 65 or older suffering from significant hearing loss [43]. Children can also develop hearing loss which can be detrimental to educational and social development. Tinnitus, or ringing in the ears, accompanies a majority of hearing conditions.

There are two main types of hearing loss: conductive and sensorineural. Conductive hearing losses are the result of physical problems with the movement of sound waves through the ear, for example, a blockage of the ear canal. Sensorineural hearing loss (SNHL) is the result of damage to the hair cells or nerves of the auditory system. Exposure to loud noise, or other acoustic trauma, ototoxic drugs (such as cisplatin), genetic predispositions, as well as a multitude of different infections and diseases of the inner ear can all lead to sensorineural hearing loss. SNHL can have many different etiologies and presents in a variety of conditions including sudden hearing loss, hearing loss due to noise exposure, autoimmune diseases and other diseases of the inner ear.

Sudden Sensorineural Hearing Loss (SSHL)

SSHL, or sudden deafness, is a common otologic disorder of uncertain etiology with spontaneous recovery of hearing in up to 65% of patients [44]. SSHL can be defined as a sensorineural hearing loss of greater than 30 dB over 3 contiguous

frequencies occurring within a period of approximately 3 days [43]. However, definitions vary based on severity, time course, audiometric criteria and the frequency of the loss. SSHL can include awakening with a hearing loss, a hearing loss noted over a few days, selective low or high-frequency loss, and distortions in speech perception. The majority of cases are unilateral and some hearing is usually recovered. SSHL has many possible etiologies including, viral infection inflammation or vascular compromise, tumours, toxins, or autoimmune disorders.

Noise-Induced Hearing Loss (NIHL)

Environmental noise is a common and preventable cause of hearing loss. NIHL can occur due to noise exposure from recreational activities (socioacusis) or, more commonly, from noise exposure in the workplace (occupational NIHL). For example, NIHL is common in construction workers and in military personnel exposed to weapon-related noise levels. NIHL develops slowly over many years of exposure. Susceptibility varies widely between individuals, but an average of 10 years or more is required for significant hearing loss to occur [43]. There is also some controversy regarding what percentage of presbycusis is a consequence of a lifetime socioacusis, and what percentage is due only to the normal process of physiologic aging.

Animals exposed to impulse noise show anatomic changes that range from distorted stereocilia of both inner and outer hair cells, to complete absence of the organ of Corti and rupture of Reissner's membrane. There are typically no reported changes to the blood vessels, spiral ligament or limbus. A few minutes after noise exposure, edema of the stria vascularis appears and may persist for several days. A cochlear inflammatory response is also initiated in response to acoustic trauma [45].

Due to their regulatory action in audition, outer hair cells are more susceptible to noise exposure than inner hair cells. Hearing loss resulting in temporary threshold shifts (TTS) is associated with decreased stiffness of the stereocilia of the outer

hair cells. This disarrayed or 'floppy' anatomy of stereocilia is assumed to affect hair cell function. Hearing loss resulting in permanent threshold shifts (PTS) is correlated with the loss, or fusion of stereocilia. As the severity of the noise exposure increases, injury can proceed from a loss of adjacent cells to complete destruction of the organ of Corti. NIHL may reflect the damage from dead hair cells, in addition to impaired, but surviving hair cells. Thus, NIHL and hair cell loss are known to show only moderate correlation [45].

Metabolic exhaustion and mechanical trauma are the two main explanations of damage from noise exposure. TTS is sometimes referred to as 'auditory fatigue', and may be due to metabolic exhaustion of the auditory system. This may explain the well-documented clinical fact that intermittent noise is less likely to produce permanent damage that continuous noise at the same intensity level. However, mechanical trauma is a more accurate explanation of the observation that the portion of the cochlea sensitive to speech frequencies (approximately 4000 Hz) is the greatest area of injury in occupational NIHL [43].

There is currently no treatment to reverse the effects of NIHL, or stop them altogether. However, recent studies have shown that the presence of glucocorticoid signalling pathways in the cochlea have a protective role against NIHL [46].

Autoimmune Inner Ear Disease (AIED)

AIED is an inflammatory condition of the inner ear. It occurs when endogenous antigens of in inner ear are identified as foreign pathogens and are attacked by the immune system. AIED was recognized in 1979 when patients with SNHL showed an improvement in hearing after treatment with corticosteroids [47]. AIED is characterized by the presence of a rapidly progressive, often fluctuating, bilateral SNHL over a period of weeks to months. The progression of hearing loss is too rapid to be presbycusis, and too slow to include SSHL. Vestibular symptoms, like vertigo and imbalance may also be present.

The exact mechanisms of inflammatory damage have yet to be elucidated. However, AIED has been successfully treated with corticosteroids for some time (Ch. 10). AIED may be caused by an association with a type I immune reaction involving an immunoglobulin E (IgE) mediated response. Inflammatory damage may also occur due to production of autoantibodies to inner ear antigen, and/or to the production of immune complexes [48].

Ménière's Disease

Also known as idiopathic endolymphatic hydrops, Ménière's disease is a disorder of the inner ear that results is vertigo, tinnitus, and SNHL. Ménière's disease was named after Prosper Ménière, who first proposed the disorder in 1861 [49]. It has been reported to affect anywhere from 15 to over 150 people per 100,000 [49]. Unilateral disease is more common, but with disease progression bilateral involvement may increase to over 40% [50].

Although the exact cause remains controversial, damage in Ménière's disease is thought to occur via the distortion of the membranous labyrinth due to the over-accumulation of endolymph. Endolymph is produced by the stria vascularis in the inner ear, and flows from the endolymphatic fluid space, through the vestibular aqueduct, and into the endolymphatic sac [51]. If any obstruction is present, endolymphatic hydrops can occur [52]. There is ongoing dispute as to whether endolymphatic hydrops are a marker or a cause of Ménière's disease.

Chapter 5. Ototoxicity

Ototoxicity is defined as damage to the ear, specifically the structures of the inner ear, including the cochlea or auditory nerve, and occasionally the vestibular system, by a toxin. Ototoxicity is generally related to bilateral, high-frequency sensorineural hearing loss and tinnitus. The hearing loss may be temporary, or asymmetrical, but is typically irreversible and bilaterally symmetrical with most toxic agents [53]. The time of onset of hearing loss is not typified. Hearing losses and other inner ear disturbances can occur after a single dose of agent, or after several weeks or months after antibiotic therapy or chemotherapy.

Ototoxicity gained clinical attention with the discovery of streptomycin in 1944. Streptomycin was used to successfully treat tuberculosis, but caused an irreversible inner ear dysfunction in a substantial number of patients [54]. These findings, along with ototoxicity of later development of other aminoglycosides, led to an increase in medical research into the pathophysiologies of ototoxicity. Today, any drug with the potential to cause toxic reactions to the inner ear is considered ototoxic. The ototoxic potential of many different specific classes of drugs has been well established. Ototoxic drugs include antibiotics, loop diuretics, and chemotherapy agents.

Aminoglycosides

Aminoglycosides are bacterial antibiotics that bind to the 30S ribosome and inhibit bacterial protein synthesis. Since their advent in 1944, many preparations have become available, such as streptomycin, kanamycin, gentamicin, neomycin and amikacin. These antibiotics may be used in combination with penicillin for staphylococcal, streptococcal, and enterococcal endocarditis. Specific groups of patients, including those with cystic fibrosis, immune deficiencies, and certain types of infectious disease, are most likely to be treated with aminoglycoside antibiotics. Aminoglycosides are the most vestibulotoxic of all ototoxic drugs, although their effects on the inner ear vary [55]. Kanamycin, amikacin and

neomycin are mostly cochleotoxic. Gentamycin affects the cochlear and the vestibular stems. Streptomycin is mainly vestibulotoxic.

Aminoglycoside toxicity affects the renal and cochleovestibular systems, with no clear correlation between the degrees of toxicity in each. Aminoglycoside-induced ototoxicity resulting in hearing loss generally begins in the high-frequencies, causing irreversible damage to the outer hair cells at the basal turn of the cochlea. Damage begins at frequencies above 4 kHz and, thus, may go unrecognized by the patient. The lower speech frequencies will inevitably become affected with progression resulting in profound deafness if the drug is continued. Ototoxic affects may be latent, as aminoglycosides are cleared more slowly from the inner ear. This latency can result in worsening of hearing loss or late-onset hearing loss after completion of aminoglycoside treatment. Audiologic monitoring for ototoxicity during and after treatment is therefore important.

Aminoglycoside hearing loss is related to the destruction of cochlear outer hair cells by disruption of mitochondrial protein synthesis and the formation of ROS; mechanisms similar to cisplatin ototoxicity (Ch. 7, Ch. 11). Aminoglycosides are thought to generate free radicals in the inner ear by activating iNOS, producing NO [56]. Oxygen radicals react with the NO to form the destructive peroxynitrite radical which has been shown to lead directly to cell death. The primary mechanism of cell death is apoptosis which is mediated by an intrinsic mitochondrial-mediated cascade. Aminoglycosides interaction with transition metals, like copper and iron, appear to potentiate the formation of free radicals. This cascade ultimately leads to permanent destruction of the outer hair cells, resulting in permanent hearing loss [6]. There is currently no treatment available aminoglycoside ototoxicity. Iron chelators and antioxidants along with gene therapy, are currently being investigated as possible protective agents against this toxicity. Animal studies have shown vitamin E, alpha lipoic acid, Ebselen, and ginkgo biloba to exert some otoprotective effect [57].

Depending on the aminoglycoside and the dosing schedule, up to 33% of adult patients may experience hearing loss with treatment. Vestibulotoxicity occurs to a lesser extent, measured in 4% of adult patients. In other countries, where antibiotics are available without a prescription, aminoglycosides have been shown to causes up to 66% of cases of severe hearing loss. On the other hand, the incidence of hearing loss from aminoglycoside treatment may be decreasing due to improvements in monitoring and increased awareness. Also, aminoglycoside ototoxicity had been shown to be less common in neonates and children than in adults, affecting approximately 2% of neonates [58].

Aminoglycoside ototoxicity has been shown to occur with larger doses, higher blood levels, or longer duration of therapy. High-risk patients include elderly patients, those with renal insufficiencies, those with pre-existing hearing problems or family history of ototoxicity, and those receiving other ototoxic medications. A genetic predisposition in the mitochondrial RNA mutation 1555A>G has also been found to be associated with aminoglycoside-induced and nonsyndromic hearing loss [59]. Careful evaluation of patient history and genetic screening has been suggested for high-risk groups [6, 53].

Other Antibiotics

Macroslide antibiotics have also shown slight tendencies towards ototoxic damage. Erythromycin was introduced in 1952 to widespread clinical use. Reports of ototoxicity were not prevalent until 1973, with sporadic cases reported since then. Affected patients tend to have other risk factors, including renal failure, or very large doses or IV administration of the antibiotic. The onset of hearing loss is usually within three days after starting treatment. The hearing loss tends to be reversible and affects the speech frequencies more than higher frequencies. Azithromycin and clindamycin are newer macroslide antibiotics with fewer gastinointestinal side-effects and a broader antimicrobial spectrum than erythromycin. Some incidences of possible ototoxic effect have appeared but reports have been sporadic and more research is needed [60].

In the 1950s, a glycopeptide antibiotic, vancomycin, was introduced. Many reports of ototoxicity, generally presenting as tinnitus, have been shown in patients with renal failure, or those receiving concomitant aminoglycoside therapy [61]. As with macroslide antibiotics, the hearing loss appears to be reversible and there is currently no conclusive evidence of ototoxicity with vancomycin alone.

Loop Diuretics

Loop diuretics are a class of medication that include different chemical groups such as sulfonamides, phenoxyacetic acid derivatives and heterocyclic compounds. Ethacrynic acid, furosemide, and bumethanide, the most effective and common diuretics, can cause ototoxicity. Loop diuretics exert their effects in the kidney at the loop of Henle. They are used to treat hypertension, cirrhosis, and heart and renal failure. Some loop diuretics, like furosemide, are also used in conjunction with antiemetics as treatment for some toxicities of cisplatin chemotherapy and should be closely monitored for ototoxicity (Ch. 7).

Ototoxicity from loop diuretics has been associated with changes to the stria vascularis affected by changes in the ionic gradients between endolymph and perilymph. These ionic changes cause edema of the epithelium of the stria vascularis, ultimately leading to a loss of hearing. In this case, ototoxicity has generally been shown to be reversible, although irreversible loss has been seen in neonates. Risk factors include dose, rate of infusion, history of renal failure, or combination with other ototoxic agents. Ototoxicity from loop diuretics manifests as immediate hearing loss, with occasional tinnitus or disequilibrium. Permanent damage has been reported for patients with renal failure, or patients receiving other ototoxic agents.

Salicylates

Acetylsalicylic acid, or aspirin, has been shown to cause low incidences of ototoxicity (approximately 1%). Aspirin is absorbed rapidly after oral administration and is hydrolyzed in the liver to its active form, salicylic acid.

Increasing amount of the drug produces tinnitus and typically a reversible sensorineural hearing loss [62]. Tinnitus is the most common adverse effect of salicylate ototoxicity. Hearing loss is generally mild to moderate and bilateral with recovery occurring one to three days after cessation of the drug. Risk factors for salicylate ototoxicity include high dose, old age, and dehydration. Salicylate ototoxicity may be treated by electrolyte monitoring and hydration, with the addition of alkaline diuresis, and is severe cases, oxygen administration and mechanical ventilation.

Quinine

Quinine, derived from cinchona tree bark, was historically used to treat malaria and fevers. Quinine ototoxicity can result in tinnitus, hearing loss, vertigo, headache, nausea, and vision loss. Hearing loss is generally sensorineural and reversible, showing a characteristic notch at 4 kHz [62].

Antineoplastic Agents

Since its discovery as an antineoplastic agent in the 1960s, the drug cisplatin has been widely used to treat many different types of neoplasms, including gynaecologic, lung, central nervous system, head and neck and testicular cancers. Cisplatin was the first member in a class of antineoplastic drugs that now includes carboplatin and oxaliplatin. Cisplatin induces many toxic side-effects, including nausea, nephrotoxicity, and ototoxicity. With the advent of hydration therapy, ototoxicity remains as the leading dose-limiting toxicity of cisplatin (See Ch. 7).

Chapter 6. Audiologic Monitoring for Ototoxicity

Audiologic monitoring for ototoxicity is usually performed for patients receiving platinum based chemotherapy treatment (cisplatin or carboplatin) and for patients receiving aminoglycoside antibiotics. The aim of audiologic monitoring is to detect ototoxic changes before hearing in the speech frequency range becomes affected, or monitor for early signs of ototoxicity. Baseline audiometric evaluations before treatment are essential for monitoring hearing. Follow-up testing during and after therapy is usually recommended to prevent possible adverse effects of ototoxic agents.

Audiologic assessment may be carried out physiologically or with behavioural testing. The main approaches to audiologic monitoring are basic audiologic assessment, high-frequency audiometry, the auditory brainstem response and otoacoustic emissions. These tests may be used separately or in combination depending on the purpose and the patient.

An audiogram is a standard way of measuring a patient's hearing threshold. The basic audiogram covers the frequency range of about 100 Hz to 8000 Hz. High-frequency audiometry tests from 8 kHz and above. The audiogram is a behavioural hearing test. Different tones are presented at a specific frequency and intensity and when the subject hears the sound they make a signal (for example raising a finger or pressing a button) to indicate that they have heard it. The lowest intensity sound that can be heard is recorded as the hearing threshold.

Behavioural audiologic tests are easily performed in adults and may even be carried out with small children using toys and visual cues. However, for small animal research physiologic testing that does not require the subject to respond is used for its ease of implementation and proven efficacy in ototoxicity models. The two foremost audiologic assessments for animal ototoxicity studies are the auditory brainstem response, and more recently, otoacoustic emissions [63-64].

Otoacoustic emissions (OAEs) are sounds generated from within the inner ear. A healthy ear may generate sounds on its own or in response to a stimulus. The sounds arise by a number of different cellular mechanisms in the inner ear but are most associated with healthy function of the cochlear hair cells responsible for audition. When hearing from hair cell damage occurs, fewer or no otoacoustic emissions can be detected. Thus OAEs are a good measure of the function of the inner ear and useful in assessing ototoxic damage to cochlear outer hair cells.

The auditory brainstem response (ABR) is a more-established method of assessing auditory function. Unlike OAEs, the ABR assesses the entire auditory pathway, not just the outer hair cells of the inner ear. For example, hearing loss from auditory neuropathy (with lesions on the auditory nerve) will not affect cochlea hair cells. This hearing loss will result in normal OAEs, with an abnormal or absent ABR. For this reason, and its well-documented use, the ABR remains the standard for audiologic monitoring in animal research.

Auditory Brainstem Response (ABR)

Since its discovery almost 40 years ago by Jewett and Williston (1971), the ABR has become a standard part of the auditory test battery. The ABR is a neurologic test of the function of the entire auditory pathway. It can be used in health care, or medical research settings to screen hearing, determine thresholds, and differentiate between different types of hearing loss.

ABR audiometry refers to an evoked electrical potential generated by a click or tone burst. This stimulus is transmitted from an acoustic transducer in the form of an insert earphone or headphone. Surface electrodes are placed at the vertex of the scalp at the ear lobes to measure the elicited response (Figure 8, Ch. 13). The ABR is characterized by a group of potentials occurring within the first 10 milliseconds (msec) following the stimulus. The amplitude, in microvolts, of the response is averaged and plotted against this time in milliseconds.

The stimulus of the ABR initiates evoked potentials from the basilar region of the cochlea. The signal then travels along the auditory pathway, through the auditory nerve, from the cochlear nucleus (CN), to the superior olivary complex (SOC), to the lateral lemniscus (LL) and inferior colliculus (IC). The group of generated potentials consists of approximately seven positive-negative waves. The first waves correspond to action potentials. Later waves may represent postsynaptic activity in the major brainstem auditory centers. The positive peaks of each waveform generally reflect the combined afferent activity in the auditory brainstem. The positive peaks of the waveforms are named with Roman numerals; waves I to VII (Figure 5) [65].

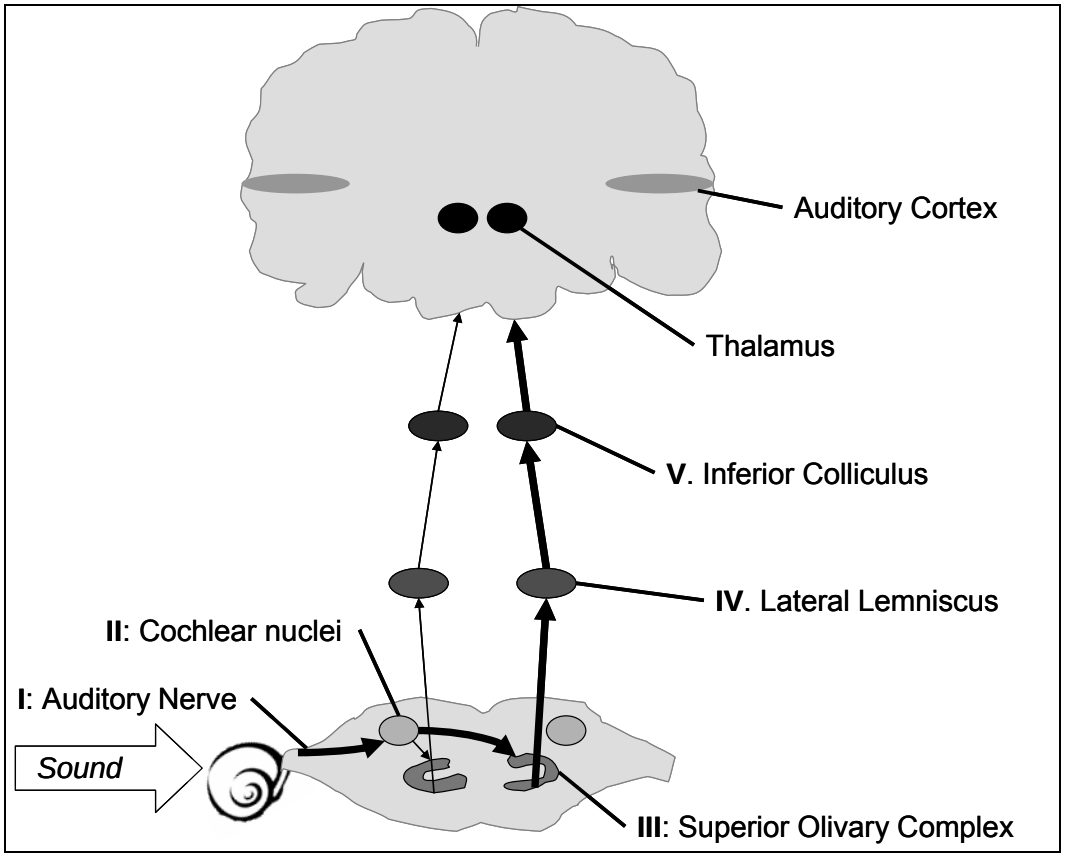

Figure 5. Diagrammatic representation of the structures involved in the auditory brainstem response and their corresponding waveforms. (McGill Auditory Sciences Laboratory 2009).

Waves I and II in the ABR represent the auditory nerve action potential of cranial nerve VIII. The response for wave I is generated by the distal eighth nerve. Wave II arises by the proximal, or brainstem portion of the eighth nerve. Wave III reflects neuronal activity in the cochlear nucleus. Wave IV occurs from neurons located in the SOC, but contributions from the cochlear nucleus and the lateral lemniscus may also be present. Wave V is believed to originate from the inferior colliculus. However, this wave most likely reflects the activity of multiple auditory structures. ABR waves I, II, and III arise from auditory pathways ipsilateral to the side of stimulation, whereas wave V reflects the activity in the midbrain auditory structures contra-lateral to the stimulus (Figure 5). The site of waves VI and VII has yet to be elucidated. It is suggested that they originated from near the thalamus or auditory cortex [65-66].

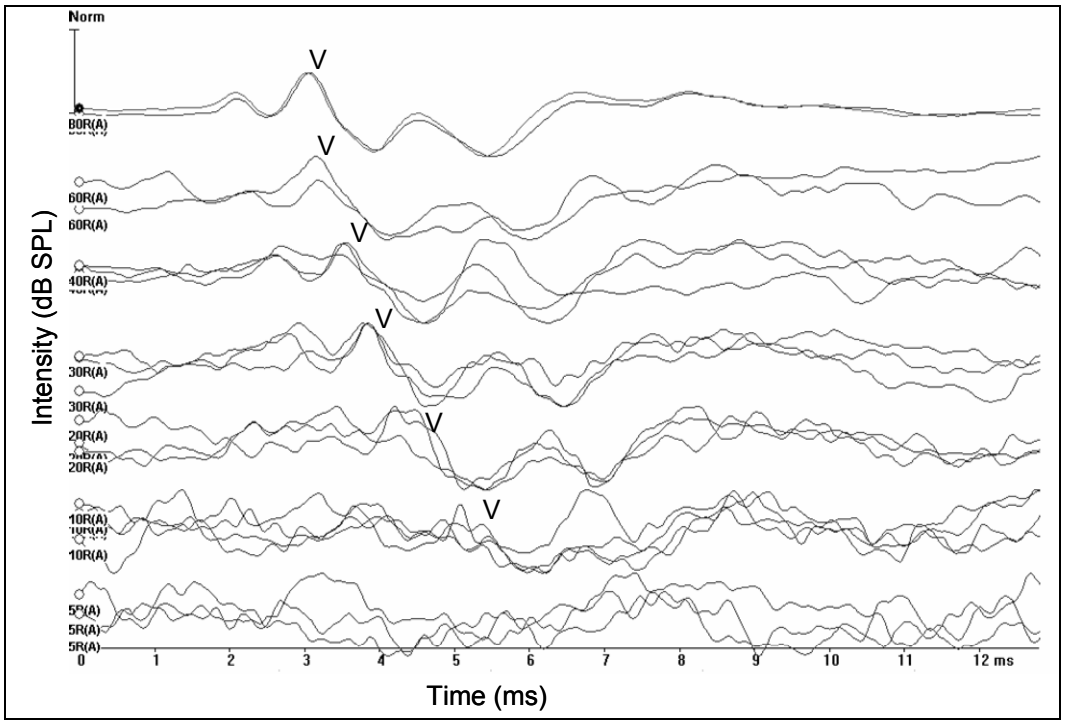

Figure 6. Example ABR recording of the right ear of a healthy guinea pig at 16 kHz with wave five (V) labelled. Here the hearing threshold is measured at 10dB. (McGill Auditory Sciences Laboratory 2009).

The ABR is a valuable tool for gaining clinical information. Wave V is the component analyzed most often in applications of the ABR (Figure 6). Wave V

and waves I to V peak latencies can be monitored for alteration in latency and amplitude. Latency from wave I to V provides information on the function of the eighth cranial nerve to the auditory brain stem. Also, the interpeak latencies between waves I and II, and I and III can provide information during surgeries to the eighth nerve [66].

The ABR may be applied in the identification of retrocochlear pathology and symptoms of eighth nerve pathology. It may be used as an effective screening tool for evaluation of an acoustic neuroma or vestibular schwannoma [67-68]. Clinical symptoms of eighth nerve pathology can include unilateral hearing loss, asymmetrical high-frequency hearing loss, or unilateral tinnitus. Retrocochlear pathology can present as latencies of wave 5, interpeak latencies of waves I-III, I-V and III-V and an absent ABR in the affected ear. In addition to retrocochlear pathologies, many factors, including the degree of sensorineural hearing loss, the asymmetry of the hearing loss or the test parameters or patient factors, may influence ABR results and should be included in analysis [65-66].

Also, although the ABR is generated by the eighth cranial nerve and auditory brainstem, the response is influenced by, and useful in detecting, conductive (middle ear) and sensory (cochlear) hearing impairment. The ABR can be influenced by subject age (those under the age of 10 months and over the age of 60 years), gender, and body temperature. The response is not grossly affected by the subject's state of arousal, including sleep, or most drugs, including sedatives and anaesthetic agents [65-66].

PART THREE: Cisplatin Ototoxicity and Otoprotection

Chapter 7. Cisplatin Ototoxicity

Although often an effective antineoplastic agent, patients can suffer extremely toxic side-effects while undergoing cisplatin chemotherapy. Clinically, at a single dose of 50mg/m^2 of cisplatin or more, patients may suffer nausea, vomiting, neuropathy, myelosuppression, nephrotoxicity and ototoxicity [69]. Rare effects of cisplatin include visual impairments, seizures, arrhythmias and pancreatitis. After treatment, continuous antiemetic therapy may be required. Renal damage occurs at the glomeruli and tubules and, like ototoxicity, is cumulative. However, nephrotoxicity has been proven to be ameliorated by hydration.

Cisplatin is typically administered as an intravenous chloride-containing solution given over 0.5 to 2.0 hours. Patients are pre-hydrated with at least 500mL of sodium chloride containing fluid. Immediately before cisplatin administration, mannitol is given parentally to maximize urine flow. Diuretics like furosemide may also be used along with parental antiemetics, such as dexamethasone and 5-hydroxytryptamine (5-Ht_3) antagonist (Ch. 9). A minimum of one litre of post-hydration fluid is usually given, and hydration varies according to dose. 'High-dose' cisplatin is loosely defined but can be considered at doses from 200mg/m^2/course.

Ototoxicity generally presents as a cumulative, dose-related, bilateral, and irreversible sensorineural hearing loss with or without tinnitus [1]. It begins in the high frequencies, but extends into the lower frequencies important for speech perception, especially with doses in excess of 100 mg/m^2 [2]. From 2 to 36% of patients complain of tinnitus [70]. If ultra-high-frequency audiometric testing is used, up to 100% of patients receiving high dose cisplatin may show some degree of hearing loss [71]. Hearing damage can occur within hours after administration, but may also not appear until several days or months after treatment. The

incidence and severity of hearing loss with cisplatin depends on the dose and amount of cycles, the rate of infusion, the renal status, dehydration and combination with any other ototoxic agents. Risk factors include dose, age, noise exposure, depleted nutritional state, exposure to other ototoxic drugs (for example loop diuretics, Ch. 5), and cranial irradiation [1, 71-72]. Cisplatin ototoxicity has been shown to affect one third of adult cancer patients, but incidences have been measured as high as 80%, with the minority suffering a significant hearing handicap [3-4]. This toxicity is much more prevalent in children, affecting from 60% up to 90% of paediatric patients [73-74].

Genetic Predispositions

Recent studies have indicated that several genetic predispositions appear to play a role in increasing or decreasing the likelihood of ototoxicity in patients treated with cisplatin. The genes encoding GSTM1, GSTT1, and GSTP1 are functional polymorphisms of the cisplatin-detoxifying enzymes glutathione-S-transferases (GSTs). Patients with a pattern of GSTT1 positive, GSTM1 positive and 105Val/105Val-GSPT1 had better hearing than those without [75]. In a study of 91 patients with osteosarcoma, cisplatin ototoxicity was also associated with the single nuclear polymorphism in the excision repair cross-complementary (CC) genotype of XPC Lys939Gln. Megalin, a member of the low-density lipoprotein family and highly expressed in kidney tissues and cells of the cochlea, has been shown to accumulate platinum-DNA adducts. Single nucleotide polymorphisms of the megalin gene have been associated with cisplatin ototoxicity susceptibility [76]. Mitochondrial mutations, specifically a rare European J mitochondrial haplogroup that is associated with Leber's Hereditary Optic Atrophy, have also been linked to cisplatin ototoxicity [77].

Mechanisms of Cisplatin Ototoxicity

In the body, cisplatin exerts its antineoplastic effects as a cell cycle non-specific alkylating-like agent that inserts into DNA and disrupts cell replication. Molecularly, cisplatin contains two chloride ligands, and two ammonia ligands

arranged in a *cis* formation around a platinum center. Specifically, the platinum complexes react causing conformational changes in DNA leading to cell death. Cisplatin uptake into the cell has been shown to occur with several different transporters, including mammalian copper transporter 1 (CTR1). Copper transporters have also been shown to regulate the export of cisplatin as well [78-80]. Once cisplatin enters a cell, cisplatin undergoes aquation, where one of its chloride ions in displaced by water. The water ligand in the resulting ion is itself easily displaced. This allows the platinum ion to interact with a basic site in DNA. Consequently, the cross-linking of two DNA bases occurs via displacement of the other chloride ligand. Cisplatin causes cross-links in DNA in different ways, causing conformational changes in DNA. DNA repair mechanisms are elicited, but apoptosis is induced when repair proved impossible.

In the cochlea, cisplatin accumulates in cellular tissues, integrates into DNA, and causes inefficient and dysfunctional protein and enzyme synthesis. However, cisplatin induced ototoxicity has long been chiefly associated with the loss of outer hair cells, beginning at the base of the cochlea and progressing towards the apex [81-82]. It has been shown that damage occurs in parallel in supporting cells in the organ of Corti, spiral ganglion cells, and cells of the lateral wall, including the stria vascularis and the spiral ligament [83-84]. This damage is induced via the production of free radicals, or reactive species (ROS), including peroxynitrite and the superoxide anion [84-85].

With the onset of cisplatin-induced free radicals, natural antioxidants of the cochlea, such as glutathione, and natural antioxidant enzymes become depleted [84]. Natural antioxidant depletion leads to an increase in free radicals and inflammatory molecules causing DNA damage, lipid peroxidation and eventual cell death [37, 42, 84]. Due to its anatomical position and its isolation, the cochlea is generally a closed system, and therefore inefficient at flushing out accumulated toxins, especially when at a rapid pace of generation [5].

Cisplatin triggers the production of these reactive species directly and through the production of inflammatory molecules [42]. Free radicals are produced by NOX and NOS in cells of the inner ear following cisplatin exposure. As mentioned, NOS produces NO; NOX catalyses the formation of superoxide radicals. NOX3, a particular form of NOX, is highly and selectively produced in the inner ear and acts a main source of free radical production [6, 37]. It has been shown to be activated by cisplatin in cochlear explants, and in the rat cochlea as early as 24 hours following cisplatin treatment [37, 86].

The free radicals generated by these mechanisms lead to mitochondria-mediated and caspase-dependent cell apoptosis and, ultimately, permanent hearing loss. The following sections outline primary molecules and cellular proteins involved in the mechanisms of cisplatin ototoxicity (Figure 7).

Superoxides are generated by various cochlear tissues, but mainly NOX3 in the inner ear. They inactivate antioxidant enzymes and are the cause of several other damaging mechanisms [87]. They can interact with NO to form peroxynitrites which nitrosylate and inactivate proteins [88-89]. They form free hydroxyl radicals that react with cellular membranes to generate highly toxic aldehyde 4-hydroxynoneal (4-HNE), leading to cell death [88-89]. An increase in 4-HNE is associated with an increase in Ca^{2+} influx into the outer hair cells resulting apoptosis [90-91]. Superoxides may also cause cytosolic migration of pro-apoptotic proteins (like Bax), leading to the release of cytochrome c from mitochondria, responsible for the activation of caspase 3 and caspase 9. In response, caspase-activated deoxyribonuclease (CAD) is activated. This causes DNA breakdown and cleavage of fodrin in the cuticular plates of injured hair cells resulting in cell destruction [92-93].

The transient receptor potential vanilloid 1 (TRPV1) protein is a member of the TRPV group of transient receptor potential family of ion channels [94]. There has been evidence of TRPV1 being used as a permanent channel by cisplatin [5]. The

activation of TRPV1 brings the added toxicity of Ca^{2+} influx and overload followed by the activation of caspases and cell death [5]. In fact, suppression of TRPV1 expression in cell cultures, as well as in the rat model, resulted in the suppression of cisplatin generated ROS, NOX3 expression and Ca^{2+} increase [86].

Cisplatin activates big conductance potassium (BK) channels in the type I spiral ligament fibrocytes of the lateral wall, disrupting the electrochemical gradient and triggering apoptosis [95]. As mentioned, the lateral wall maintains the ionic environment of the endolymph which has a higher potassium and lower calcium concentration than the surrounding perilymph and maintains a high resting potential (Ch. 2). Cisplatin induces persistent activation of BK channels, leading to potassium efflux which decreasing intracellular potassium and causes a loss of osmotic pressure and ionic concentration, which in turn triggers pro-apoptotic nucleases and cleavage of caspases resulting in cell death [95-97].

Transcription factors are known to play a role in cisplatin ototoxicity. NFkB is a widespread protein that controls the transcription of DNA, and is involved in a multitude of complex cellular responses. NFkB and iNOS have been shown to be up-regulated in the stria vascularis and the spiral ligament of cisplatin-treated mice [98]. Consequently, in vitro experiments have shown that NFkB is essential for survival of immature auditory hair cells [99]. Increased NFkB staining has been shown in the organ of Corti, the spiral ligament and the stria vascularis in cisplatin-treated cells in vitro and in the rat cochlea [42]. In addition, NFkB and pro-inflammatory cytokines, including TNFα, IL-1, and IL-6, are up-regulated by cisplatin treatment in vitro and in the rat cochlea [34]. STAT1 is a member of the Signal Transducers and Activators of Transcription family of transcription factors. It is known to mediate cell death in exposure to ROS [100-103]. This transcription factor has also been shown to mediate cell death in exposure to UV radiation, TNFα and DNA damage [103-104].

Many studies have attempted to attenuate the production of reactive species with the administration of neutralizing antioxidants [84]. Unfortunately, many of these products have either interfered with the antineoplastic properties of cisplatin, or caused unwanted effects in human studies [6]. Currently, there are no drugs, or available treatments against cisplatin-induced ototoxicity [5].

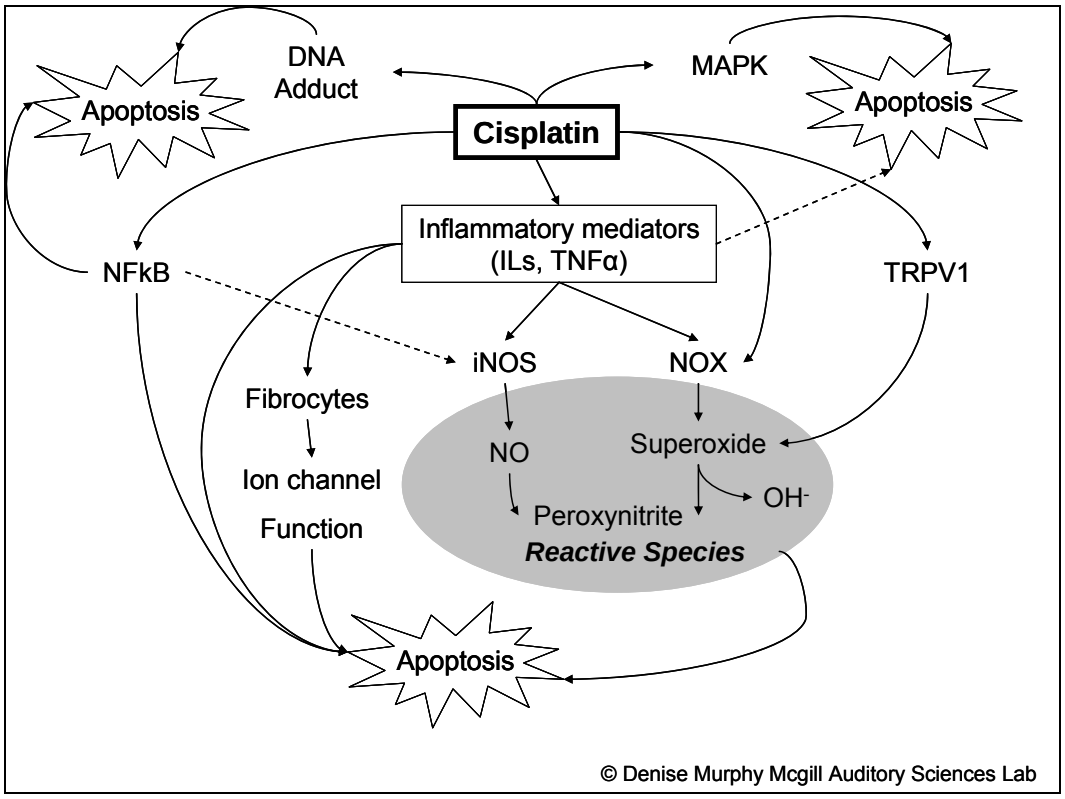

Figure 7. Generalized mechanisms of cisplatin-induced ototoxicity in the inner ear, McGill Auditory Sciences Laboratory 2009.

Chapter 8. Otoprotection

In addition to the cochlear immune response, the inner ear has other natural mechanisms of protection against stresses such as those caused by cisplatin. Endogenous protective molecules include glutathione and the antioxidant enzymes, heat shock proteins, and adenosine A1 receptors [86]. These protective molecules are all present in the cochlea and are up-regulated in response to oxidative stress. However, damage from cisplatin often overwhelms these intrinsic defence mechanisms, resulting in ototoxicity.

Naturally, antioxidant compounds were first researched, and still continue to be investigated, as possible otoprotectants against ototoxicity. Thiol-containing compounds represent the bulk of investigated otoprotectants to date. Steroids are more recently being explored for their potential as otoprotective agents.

Thiol-Containing Compounds

Thiols, or mercaptans, are compounds that contain a functional group composed of a sulphur-hydrogen bond. They are considered antioxidants in that most are electrophilic and can act as free radical scavengers. Thiols present as useful otoprotective agents because of the high affinity of sulphur for platinum. In fact, thiols have so far been the major focus of otoprotection in the clinical setting. Many thiol antioxidants have been shown to offer some protection against cisplatin ototoxicity. These thiols include sodium thiosulfate, D- and L-methionine, lipoic acid, amifostine, N-acetylcysteine, glutathione ester, methylthiobenzoic acid and diethyldithiocarbamate [6]. The following paragraphs describes the major groups of thiol-containing drugs for otoprotection.

Sodium Thiosulfate is directly incompatible with cisplatin. It acts to neutralize cisplatin by forming a complex that is eventually secreted by the kidney, reducing cisplatin's ototoxic effect, as well as its antineoplastic potency [105]. Assessment by ABR showed that administration of STS with cisplatin protected against cisplatin-

induced ototoxicity [106]. To date, two other studies in guinea pigs have demonstrated the effect of sodium thiosulfate against cisplatin ototoxicity. Wang *et al.* showed that perilymphatic perfusion of STS prevented cisplatin ototoxicity [107]. Wimmer *et al.* showed no protection of STS when applied by osmotic pump to the round window membrane [105]. Both studies used an ototoxicity model of low dose cisplatin over 5 days, with a higher total dose used by Wimmer *et al.* Since perilymphatic perfusion is impractical for clinical use, and due to the neutralising effect of systemic STS on cisplatin's antitumor activity, STS remains an inefficient treatment modality for cisplatin ototoxicity.

Chemoradiation protocols with intra-arterial cisplatin include thiosulfate as a protective agent. However, thiosulfate delivered in this fashion may not provide protection. In a recent clinical study of 146 patients, 23% of ears were still found to require hearing aids after cisplatin, despite treatment with thiosulfate [108].

Both isoforms of the sulphur-containing amino acid methionine may act to reduce cisplatin ototoxicity [109-110]. However, it is not clear whether or not methionine has a negative affect on the antineoplastic activity of cisplatin [111-112]. L- and D-methionine have been shown to act as free radical scavengers and prevent the induction of NOS [110, 113-114]. Methionine has also been found to readily pass through the round window membrane and travel to the lateral wall and perilymph of the cochlea and the organ of Corti [115]. L- and D-methionine appear to be promising otoprotectants, although their defence against cisplatin is still unclear [112].

Amifostine, also known as WR-2721, or WR-1065, was first developed for use as a radioprotectant by the US Military [116]. Since then, it has been investigated for its cytoprotective properties against cisplatin chemotherapy. Amifostine has been found to protect against ototoxicity. However, it leads to neurotoxicity in the hamster [117]. There have also been a small number of clinical trials testing amifostine as a protective agent. Adult patients experienced severe ototoxicity

following cisplatin administration despite pre-treatment with amifostine [118]. Children treated with cisplatin and amifostine also showed no protection against ototoxicity [119-120]. In 2008, amifostine was not considered to afford any otoprotection against ototoxicity by the American Society of Clinical Oncology [121].

N-Acetylcysteine (NAC) is a drug used mainly as a mucolytic agent and in the management of acetaminophen overdose. NAC, administered systemically or transtympanically presents as a possible otoprotectant against cisplatin ototoxicity. Intravenous NAC given before and after cisplatin in rats demonstrated significant preservation of ABR thresholds [122]. Transtympanic administration of NAC in saline and Ringer's Lactate showed a preservation of DPOAEs after cisplatin in guinea pigs [123].

Glutathione is an endogenous antioxidant that participates directly in neutralizing free radicals and maintaining exogenous antioxidants, such as vitamin E. It is also fundamental in numerous metabolic and biochemical reactions, such as DNA synthesis and repair, protein synthesis and enzyme activation. Glutathione, along with antioxidant enzymes glutathione peroxidase and glutathione reductase, have been shown to be depleted after ototoxic doses of cisplatin in animal studies [124]. With a glutathione-supplemented diet, rats were showed some protection against gentamicin ototoxicity [125]. When glutathione or glutathione ester was administered to cisplatin-treated rats, glutathione was not significantly protective, but glutathione ester showed an otoprotective effect. Additionally, this protection decreased as the dose of glutathione ester increased, which suggested a possible toxicity [126]. Ebselen is a glutathione peroxidase mimic and is also an excellent scavenger of peroxynitrite radicals. It has been shown to protect against lipid peroxidation in the presence of glutathione, or other thiols [127-129].

Other Antioxidants

Sodium salicylate is the sodium salt of salicylic acid and is used as an analgesic and antipyretic. Given systemically to tumour-infected rats, it has been shown to afford protection against ototoxicity and nephrotoxicity without affecting the antineoplastic efficacy of cisplatin [130]. Based on more studies in rats, it is suggested that sodium salicylate may provide an antioxidant effect that antagonizes cisplatin ototoxicity [131]. Sodium salicylate was also found to afford partial protection of the outer hair cells against cisplatin ototoxicity in guinea pigs [132]. However, especially in high doses, salicylate has also been found to cause ototoxicity (Ch. 5) [133-135].

Tocopherols (Vitamin E) are a family of fat-soluble antioxidants. Of these, α-tocopherol is the most abundant and has been the most studied. Following systemic treatment, α-tocopherol has been shown to reduce cisplatin outer hair cell damage and ABR threshold elevations in rats [136]. In some studies it also blocked lipid peroxidation in the cochlea, and prevented outer hair cells death accompanied by ABR threshold elevations in guinea pigs [137]. However, in another study in guinea pigs, α-tocopherol treatment only offered partial protection from cisplatin ototoxicity. In this study, greater protection was obtained when α-tocopherol was combined with other, thiol-containing compounds [138]. Trolox® (Oxis), a water-soluble form of vitamin E, was found to be effective against cisplatin ototoxicity when applied topically to the round window membrane of guinea pigs [139]. In 2004, a clinical trial provided diets supplemented with vitamin C, vitamin E and selenium to 48 patients during cisplatin chemotherapy but found no protective effect with the added vitamins [140].

Adenosine receptors are a class of plasma membrane receptors involved in inflammatory and immune processes. Specifically, endogenously expressed A_1 adenosine receptors (A_1ARs) have been shown to afford protection against oxidative damage of cisplatin [141]. In chinchilla cochlea, localized application of A_1AR agonist resulted in an increase in antioxidative enzymes glutathione

peroxidise and superoxide dismutase [142]. Interestingly, adenosine receptors have also been shown to be up-regulated by cisplatin, perhaps as a compensatory mechanism by the cochlea, to counter the toxic effects of increased ROS generated by cisplatin [143].

Steroids

Corticosteroids, specifically glucocorticoids, have gained attention as possible otoprotectants against ototoxicity. Corticosteroids have a lengthy clinical history as treatment for a variety of inner ear disorders, such as Ménière's disease, and sudden sensorineural hearing loss. More recently, potent anti-inflammatories, such as dexamethasone, have demonstrated their ability to protect the cochlea from drug-induced ototoxicity (See Ch. 9).

Chapter 9. Glucocorticoids

Corticosteroids have a long and well-documented medical history. A class of steroid hormones, they are involved in a wide array of physiologic systems like stress response, immune response and the regulation of inflammation. There are two classes of corticosteroids: mineralocorticoids and glucocorticoids. Mineralocorticoids, such as aldosterone, mainly promote sodium retention in the kidney, controlling electrolyte and water levels. Synthetic and endogenous glucocorticoids are multifunctional. They can control carbohydrate, fat and protein metabolism and act as anti-inflammatories by numerous mechanisms, including preventing phospholipids release and decreasing eosinophil action. Glucocorticoids may also be used to prevent nausea, often in combination with the 5-HT_3 antagonists.

Glucocorticoids are generally safe compounds. However, in very large doses, or with prolonged use, they may cause some undesired side-effects, such as Cushing's syndrome (hypercorticism). Because of their immunosuppressive effects, glucocorticoids may also cause impaired wound healing or ulcer formation. Long-term or over-use of glucocorticoids can also lead to side-effects such as hyperglycemia, skin fragility, osteoporosis, adrenal insufficiency, anovulation, glaucoma, and cataracts.

Mechanisms of Action

Subcellularly, corticosteroids have an anti-inflammatory effect by activating glucocorticoid receptors, which interact with inflammatory transcription factors resulting in suppression of pro-inflammatory molecules [144]. At the cellular level, corticosteroids reduce the quantity of inflammatory cells, for example eosinophiles, T lymphocytes, mast cells, and dendritic cells. In fact, the degree of inflammatory suppression correlates with the tissue concentration of steroid [8].

Glucocorticoids are known to penetrate the cell membrane and bind to soluble cytoplasmic receptors, forming a glucocorticoid-receptor complex [145]. This complex migrates to the nucleus and binds to specific DNA sites, termed glucocorticoid response elements. Here they activate and suppress expression of certain genes that are cell type-dependent, thus producing specific reactions such as anti-inflammatory responses (Ch. 11) [41]. The up-regulation of genes encoding proteins with a wide range of anti-inflammatory effects is termed transactivation. Glucocorticoids also act in an opposite mechanism termed transrepression. In this case, the activated receptor interacts with specific transcription factors, like NFkB, and prevents the transcription of specific genes. Thus, glucocorticoids are able to prevent the transcription of pro-inflammatory genes, including those encoding IL-1 and TNFα.

Glucocorticoids have also been shown to exert numerous rapid actions that are independent of the regulation of gene transcription. Binding of glucocorticoid to the GR is associated with vasorelaxation, the mediation of lymphocytolysis, and the inhibition of inflammatory prostaglandins [146-150].

Pharmacokinetics

There are a variety of synthetic, highly potent glucocorticoids, differing in pharmacokinetics and pharmacodynamics that have been created for therapeutic use. Glucocorticoid potency and duration of effect varies depending on the steroid and route of administration. Cortisol (hydrocortisone) is the standard of comparison for glucocorticoid potency, which is generally based on biological half-life. Approximate, systemic properties of some common steroids are shown in Table 1.

Type	*Drug*	*Relative GC Potency*	*Biologic Half-Life (h)*
Short-acting	Cortisol	1.0	8-12
Intermediate-acting	Prednisone	4.0	18-36
	Methylprednisone	5.0	18-36
	Triamcinolone	5.0	18-36
Long-acting	Dexamethasone	25	36-54

Table 1. Approximate properties of common glucocorticoids, modified from Liapi [151].

Dexamethasone

Dexamethasone is a potent, synthetic member of the glucocorticoid class of steroids. It is an anti-inflammatory and immunosuppressant. It is approximately 20 to 30 times more potent than hydrocortisone, and 4 to 5 times more potent than prednisone or methylprednisone [152] (Table 1). It has many medicinal uses, including the treatment of inflammatory and autoimmune conditions [153]. There are other steroids that have greater potencies than dexamethasone. For example, triamcinolone acetonide, a more potent type of triamcinolone, is known to have greater potency. However, dexamethasone is currently the most potent steroid with established clinical otologic use (Ch. 10)

In oncology, dexamethasone is given to cancer patients to counteract certain side-effects of their chemotherapy. Dexamethasone acts as an antiemetic by augmenting the effect of 5-HT_3 receptor antagonists, for example ondansetron. Dexamethasone is used in certain haematological malignancies, especially for treatment of multiple myeloma where dexamethasone is given alone, or with thalidomide (thal-dex), or a combination of Adrinamycin (doxorubicin) and vincristine (VAD). In primary and metastatic brain tumours, dexamethasone is also used to counteract the development of edema. It is also given in cord compression to decrease the compression of a tumour on the spinal cord. Dexamethasone has many other medical uses, including use in treatment of

cerebral and pulmonary edema, as well as in a diagnostic context due to its ability to suppress the natural pituitary-adrenal axis.

Clinical Otologic Use

Corticosteroids are frequently used in a wide range of otologic conditions due to their anti-inflammatory properties. Glucocorticoids have been prevalently used in the treatment of many different otologic disorders. Historically, they have been given systemically as treatment for many cochlear disorders, and in some cases this remains the gold standard of treatment. More recently, steroids are being given transtympanically as an alternative to systemic therapy (Ch. 10). Systemic treatment with steroids has been focused mainly on Bell's palsy, sudden sensorineural hearing loss (SSHL) and other inflammatory conditions of the inner ear.

Corticosteroids are used to treat Bell's palsy due to either their possible immune regulating effect on a potential viral agent, or their anti-inflammatory effect in reducing neural edema, or both [8]. There have been a number of clinical studies that support the use of steroids to treat facial paralysis. Austin *et al.*, reported improved facial nerve recovery for Bell's palsy patients receiving corticosteroids compared with placebo [154]. Ramsey *et al*. concluded that corticosteroid therapy significantly improves facial recovery by 17% [155]. In addition, a recent double-blind, placebo-controlled randomized trial including roughly 500 Bell's palsy patients concluded that early corticosteroid treatment significantly improves the chance of complete facial recovery [156].

For decades, steroids alone, or in combination with other therapies, have been used to treat SSHL with conflicting results [8]. A study using oral steroids demonstrated significant hearing recovery, while a different clinical trial failed to show a benefit of systemic steroids in SSHL [157-158]. After surveying randomized controlled studies, Haberkamp and Tanyeri concluded that systemic steroid therapy is the best treatment modality for SSHL [159]. In fact, many authors have

deemed systemic steroids to be the standard treatment for SSHL [157]. The success of this treatment is attributed to the potent anti-inflammatory action of steroids. In an effort to achieve higher inner-ear steroid concentration while avoiding systemic side-effects, SSHL treatment has more recently shifted to IT therapy which has been shown to be more beneficial (Ch. 10) [160-162]. However, the variability of study parameters makes interpretation and comparisons difficult. Recent reviews of all randomized controlled trials where steroids were used to treat SSHL concluded that the success of steroid therapy in the treatment of SSHL remains unclear [163-164].

Chapter 10. Transtympanic Therapy

Intratympanic (IT), or transtympanic, therapy is an emerging technique in otology [165]. The direct administration of medication via a transtympanic perfusion has been used as a treatment delivery for many conditions of the inner ear, including for drug-induced ototoxicity. The first treatments used to control diseases of the inner ear, aminoglycosides for Ménière's disease, and steroids for SSHL, were delivered systemically. Unfortunately, with the systemic route of delivery, there is variable penetration into the inner ear due to the blood-labyrinthine barrier of the cochlea, and the potential for undesirable systemic side-effects. IT drug delivery avoids most pitfalls associated with systemic delivery and has become a routine strategy for treating inner ear disease [166].

The modern use of IT therapy evolved from the prior use of systemic drugs delivered to achieve the same effect. Systemic therapy consists of dosing medication via the oral, intravenous, or intramuscular route, with the intention of affecting inner ear function. The concept of IT therapy may have first been used in the 19th century, when Jean Marie Gaspart Itard removed purulent secretions from the middle ear through tympanic membrane perforations with the aid of solvents and cleansing solutions [12]. It is Harold Schuknecht who is credited with the first modern attempt at IT treatment. In 1956, he proposed the IT injection of streptomycin for the treatment of Ménière's disease.

Between this first report by Schuknecht in 1956 and the 1970s, there is almost no mention of IT drug delivery, other than the use of antibiotics for ear disease. Throughout the 1970s and 1980s, there were reports of IT aminoglycoside application for Ménière's disease, as well as few reports of IT application of other medications. In 1973 Bryant described the successful use of intratympanic steroids in a patient with facial paralysis [167]. There were also several other reports that described the use of intophoresis (electrical current) to deliver steroids into the middle ear [168-169]. By the 1990s, the use of IT therapy became more common.

The use of IT therapy is currently widespread, including steroids or aminoglycosides for Ménière's disease, steroids for hearing loss, and lidocaine for tinnitus [12]. It is a cost-effective, office procedure that can be done under local anaesthesia, and is typically well-tolerated [170]. The technique enables rapid, efficient and simple delivery of medication to the middle and inner ear without the systemic risks of other routes of administration. It allows for an increase in the level of concentration of the drug to where it is most needed. In unilateral conditions, the affected ear may be selected for treatment, avoiding any interventions with the healthy ear. IT therapy may also be used as salvage therapy when systemic treatment fails.

Due to its simplicity, there are rarely any disadvantages to IT therapy. Some infrequent complications include pain, vertigo upon drug application, otitis media, a complete perforation of the tympanic membrane, and perhaps hearing loss from these complications. Other potential drawbacks of IT delivery include anatomic barriers to absorption at the round window membrane, loss of drug down the Eustachian tube, and the variable or unknown pharmacokinetic profiles of drugs delivered via this route [166].

The primary route of entry of drugs into the inner ear is through the round window membrane. The drug's molecular weight, as well as physical properties of the round window membrane, may have an effect on diffusion [171]. In the minority of cases, the round window membrane can be partially obscured by mucosal membranes, which can interfere with delivery of medication to the round window membrane [172]. Drugs may also enter the inner ear through the oval window, vasculature, or lymphatics [173].

Drug solute concentration increases in the endolymphatic and perilymphatic spaces after passing through the round window membrane [174]. Once medication has been absorbed, it is distributed throughout the inner ear fluids mainly by interscalar exchange, rather than longitudinal transport past the helicotrema [171].

Concentrations of medications, especially steroids, achieved in the inner ear fluids after IT treatment are much higher than concentrations achieved by systemic administration [175-176]. Perfusion was further enhanced in an animal model by the use of a facilitating agent (Histamine) to increase round window membrane permeability [177].

Surgical Techniques

Many varied protocols exist for IT injection of medication for otologic disease. Usually, the patient's ear is anaesthetized and approximately 0.3 to 0.5 mL of medication is injected into the middle ear space near the region of the round window. Tympanostomy tubes have also been used to maintain an open route of delivery for repeated drug applications. Inert materials (for example Gelfoam, or MicroWicks) placed at the round window have also been used as a vehicle for passive drug delivery across the tympanic membrane and into the inner ear [178]. There are many different types of IT techniques available including, blind IT injection of medication, injections onto an object placed in the round window niche, and different types of sustained release devices. As with the multiple techniques, there exist numerous applications of IT technology in treating inner ear disease.

Clinical IT Application of Steroids

Over the last decade, there has been a proliferation of IT therapy for the steroidal treatment of Ménière's disease, sudden sensorineural hearing loss, and autoimmune inner ear disease [179]. Two of the most prevalent steroids in use are dexamethasone and methylprednisone.

There is significant variability in the uses and outcomes of IT therapy for Ménière's disease and SSHL. The dose and type of steroid used differs between studies. As mentioned, there are two main steroids in clinical IT use: methylprednisone, and dexamethasone. Steroid concentrations used range from 1 to 24 mg/mL of dexamethasone or up to 80 mg/mL of methylprednisone [170, 179]. It

appears that for most studies, the steroid dose was dependent on what was available in the market, instead of maximum doses. For example, dexamethasone was removed from the market in late 2000 and can now only be produced as a compound. Consequently, its clinical use diminished [179]. Methylprednisone's use as a common IT steroid may stem from an animal study that showed higher concentrations of methylprednisone in the endolymph after IT injections [176]. A more recent study, reinterpreting these absorption results allowing for sampling effects, showed that dexamethasone absorption into the stria and surrounding tissues was much more rapid than methylprednisolone [180]. This demonstrated dexamethasone as a more efficient steroid for intratympanic perfusions.

Another source of variation in studies and outcomes is the disease stage at the time of IT treatment. Most studies mentioned agree that early treatments are associated with better outcomes. Ideally, steroid perfusion should be performed at an early stage of disease, before any permanent damage has occurred. However, in clinical practice, patients are rarely seen at the early stages of Ménière's disease, or to a lesser degree SSHL [179].

During the 1990s, the number of surgical procedures for Ménière's, including labyrinthectomy and endolymphatic sac surgery, decreased [181]. At the same time, the use of IT gentamicin therapy increased rapidly. By 1999, it had become the most frequently performed procedure for the treatment of Ménière's disease [181]. The aim of aminoglycoside treatment is the chemical ablation of the vestibular system in an attempt to reduce the frequency and severity of vertigo attacks [178]. In an attempt to reduce the symptoms of Ménière's disease while keeping the vestibular and cochlear systems intact, treatment has shifted to the use of IT steroids.

Many studies have focused on the use of IT dexamethasone for treatment of Ménière's disease [170]. The studies vary greatly in steroid dose, duration, method of application, and patient selection [8]. However, most used dexamethasone, and

report improvements in vertigo and tinnitus [182-184]. A recent placebo-controlled study used dexamethasone for 5 consecutive days and followed patients for up to 2 years and showed substantial control of vertigo in 82% of treated patients [185]. The most recent retrospective study of dexamethasone reported 91% "acceptable" vertigo control [186]. Comparisons and conclusions between studies remain difficult due to differences such as the dose, schedule and time course of treatment, and the use of placebo, which is important concerning the fluctuating course of Ménière's disease symptoms.

For SSHL, IT application of dexamethasone has become a common treatment, especially for patients unresponsive to oral steroids, or in those with medical contraindications to systemic steroids, like immunosuppression, diabetes mellitus, and peptic ulcer disease [178]. IT administration of steroids has also been reported to be successful salvage therapy after failure of systemic steroids [160-162, 187]. However, in two recent studies, the addition of IT administration to systemic steroid therapy did not demonstrate any hearing recovery [188-189]. A placebo-controlled, double-blind study demonstrated that patients treated with a combination of IT dexamethasone and systemic therapy had a higher likelihood of recovery than patients given systemic steroids only [190]. Studies have demonstrated improvement with different steroids, most commonly methylprednisolone and dexamethasone [191-192].

Autoimmune inner ear disease (AIED) is similar to Ménière's disease, with more rapid progression and bilateral symptoms. Studies have shown that inflammation plays a major role in AIED, specifically targeting the epithelium [193-195]. Steroids exhibit their effect by inhibiting the secretion, or generation, of pro-inflammatory cytokines by the endothelial cells [33]. Recent animal studies have also supported the use of steroids to improve and restore normal stria vascularis function in autoimmune hearing loss [196]. As with SSHL, IT steroid therapy is a main treatment modality for AIED patients, especially those who have medical contraindications to systemic steroids.

Chapter 11. Dexamethasone and Cisplatin Ototoxicity

As discussed, there have been several investigations of various chemoprotective antioxidants as otoprotectants against cisplatin ototoxicity. Antioxidants offer downstream protection by scavenging free radicals and, hopefully, stemming exponential damage from reactive species. However, the targeted affect of antioxidants against cisplatin ototoxicity may be 'too little, too late', or simply not potent enough to protect against cisplatin. Glucocorticoids are potent anti-inflammatories, have a well known clinical history of transtympanic use, and do not interfere with the antineoplastic activity of cisplatin. In fact, dexamethasone is given as antiemetic treatment against cisplatin-induced nausea (Ch. 9). Clinically, dexamethasone is one of most popular glucocorticoids, used for decades to suppress immune response and inflammation [41].

To date, detailed mechanisms of glucocorticoid action in the inner ear remain to be elucidated. As mentioned, glucocorticoids have been shown to limit the formation of ROS in the inner ear [9-10]. Dexamethasone down-regulates the production of pro-inflammatory cytokines, such as IL-1 and TNFα, by macrophages and monocytes, as well as mediator-induced expression of endothelial adhesion molecules, such ICAM-1 [41, 197-199].

Dexamethasone is thought to suppress the inflammatory response of spiral ligament fibrocytes [41, 199]. This may directly act to counteract the effects of cisplatin on spiral ligament fibrocytes and thus prevent subsequent cochlear malfunction (Ch. 7). Spiral ligament fibrocytes may play a major role, as the spiral ligament shows strong expression of glucocorticoid receptors [200]. In fact, glucocorticoid receptor expression in the inner ear has been reported by several investigators, with high concentrations observed in the spiral ligament [200-204].

IT glucocorticoid therapy may be effective when spiral ligament inflammation is involved in cochlear dysfunction [41]. In animal studies, inflammatory cells were

identified not only in scala tympani and around cochlear vessels, but also in the inferior portion of spiral ligament [41]. The molecular weights of cytokines, such as TNFα, are low enough to reach the perilymph via the round window membrane. Cells bordering the spiral ligament from the scala tympani contain micropores that allow the perilymph free access to the spiral ligament [205]. Because the molecular weight of dexamethasone is also low enough to permeate the round window membrane, it is likely that dexamethasone applied to the middle ear cavity can reach spiral ligament fibrocytes via the same route [41].

There is also evidence that dexamethasone is a potent inhibitor of the activation of transcription factors such as NFkB. This may account for the bulk of anti-inflammatory actions and the potential otoprotection of dexamethasone in the inner ear [198]. Animal studies have also shown glucocorticoids to have a protective benefit against aminoglycoside ototoxicity, which is thought to have similar pathology to cisplatin ototoxicity [206-207]. In addition, dexamethasone was also shown to increase the expression of active and passive Na^+/K^+ channels and of active water channels (aquaporins) in the endolymph surrounding tissues, potentially helping to maintain ion concentrations essential for transduction processes and, therefore, for proper cochlear function [208-210].

Three recent animal studies have all demonstrated a significant otoprotection against cisplatin ototoxicity with IT dexamethasone [211-213]. Due to its proven safety, anti-inflammatory properties, and its ability to suppress reactive species in the inner ear, IT dexamethasone presents as a safe, simple and effective treatment modality against cisplatin ototoxicity.

Part Four: Animal Study

Chapter 13. Experimental Design

Animal Subjects

Female, albino, Hartley guinea pigs (*Cavia porcellus*) purchased from Charles River Lab (Wilmington, MA) were used in this study. The guinea pigs were adults with a weight ranging from 400 to 800 g. Subjects were kept in standard housing until injected with cisplatin when they were isolated with cisplatin-treated animals only. Access to food pellets and water was provided to all animals *ad libitum*. Once treatment with cisplatin began, animals were also supplemented with additions of hay approximately twice a day. The animals were monitored daily for signs of pain and weight loss.

Assessment of Ototoxicity: Auditory Brainstem Response (ABR)

The Auditory Brainstem Response (ABR) was recorded at specific frequencies using the Smart EP device (Intelligent Hearing Systems). The first ABR test was carried out before any procedures were performed on animal subjects to ensure that all animals had functional hearing at baseline. Post-treatment ABR recordings were performed on all animal study groups 72 hours (Day 3) after treatment with cisplatin. In the case of the Dexamethasone controls, post-treatment ABR recordings were taken 72 hours (Day 3) after Dexamethasone injection. In addition to this, and depending on the study group, subsequent ABR tests were completed 14 days after cisplatin (Week 2), and 30 to 50 days after cisplatin (One Month).

The acoustic stimuli were presented through High-frequency transducers at 8, 16, and 25 kHz. The stimuli consisted of tone bursts of 8, 16, and 25 kHz tone burst (Blackman envelope), presented at a rate of 39.1 bursts/second with alternating polarity. For each given frequency, the tone burst intensity started at 80 dB SPL and decreased in steps of 20, 10 and 5dB, respectively, until the threshold was reached without going lower than 0dB. Depending on the frequency and the level of hearing threshold, post-treatment ABR recordings were measured as high as

110dB. The ABR threshold was defined as the lowest intensity for which a repetitious response could be recorded at least three times.

The electrical activity was recorded using needle electrodes placed subdermally. The negative, ground, and positive electrodes were placed on the pinna of the ear for which the ABR was recorded, the pinna of the contralateral ear, and animals' vertex, respectively. The response was amplified 100,000 times, band filtered in the range of 100 to 1500 Hz (including Line filter) and averaged 1600 times. Following the completion of the baseline ABR test, animals were hydrated subcutaneously with 10 mL sterile saline.

Drugs and Anaesthesia

Anaesthesia for ABR recordings and IT injections was administered using a self-contained, closed-circuit isoflurane delivery system. Through a large, rodent-sized snout mask, isoflurane mixed with oxygen was administered. Four different concentrations of dexamethasone product were used throughout the studies. Dexamethasone solution at 40 mg/mL and 24 mg/mL was created by mixing dexamethasone-21-phosphate (MPBiomedicals, Sigma-Aldrich Canada) with 0.9% sterile saline. Dexamethasone at concentrations of 10 mg/mL and 4 mg/mL were purchased as commercially available IV solutions (Sandoz Canada).

IT Drug Delivery

IT injections of dexamethasone or 0.9% saline were performed under anaesthesia under an operating microscope. After the tympanic membrane was visualized using an aural speculum, a sterile polyethylene microcatheter connected via a sterile 30-gauge needle to a 1mL syringe (BD Pharmaceuticals) was passed through the tympanic membrane. Approximately 0.05 to 0.15 mL (enough to fill the middle ear) of either solution was injected into the middle ear space. This injection was carried out on the day before cisplatin administration, as well as the day of cisplatin administration. (A third injection was attempted in the bilateral studies if less than 0.02 mL total had been delivered).

Cisplatin Delivery

Intraperitoneal cisplatin (Mayne Pharma Canada Inc.) at a concentration of 1 mg/mL, combined with at least 5 mL sterile saline, was injected with a 26-gauge needle on a 10 cc syringe as a bolus dose, followed by a subcutaneous bolus of 10 mL sterile saline for hydration. This administration was done in the morning and afternoon for a total of two administrations in one day. For the following three consecutive days, subjects also received two subcutaneous injections of sterile saline per day for hydration. At the conclusion of the final ABR recording, guinea pigs were deeply anesthetised with isoflurane and oxygen and euthanized by CO_2 gas in a specialized chamber. No parts were preserved for histological evaluation.

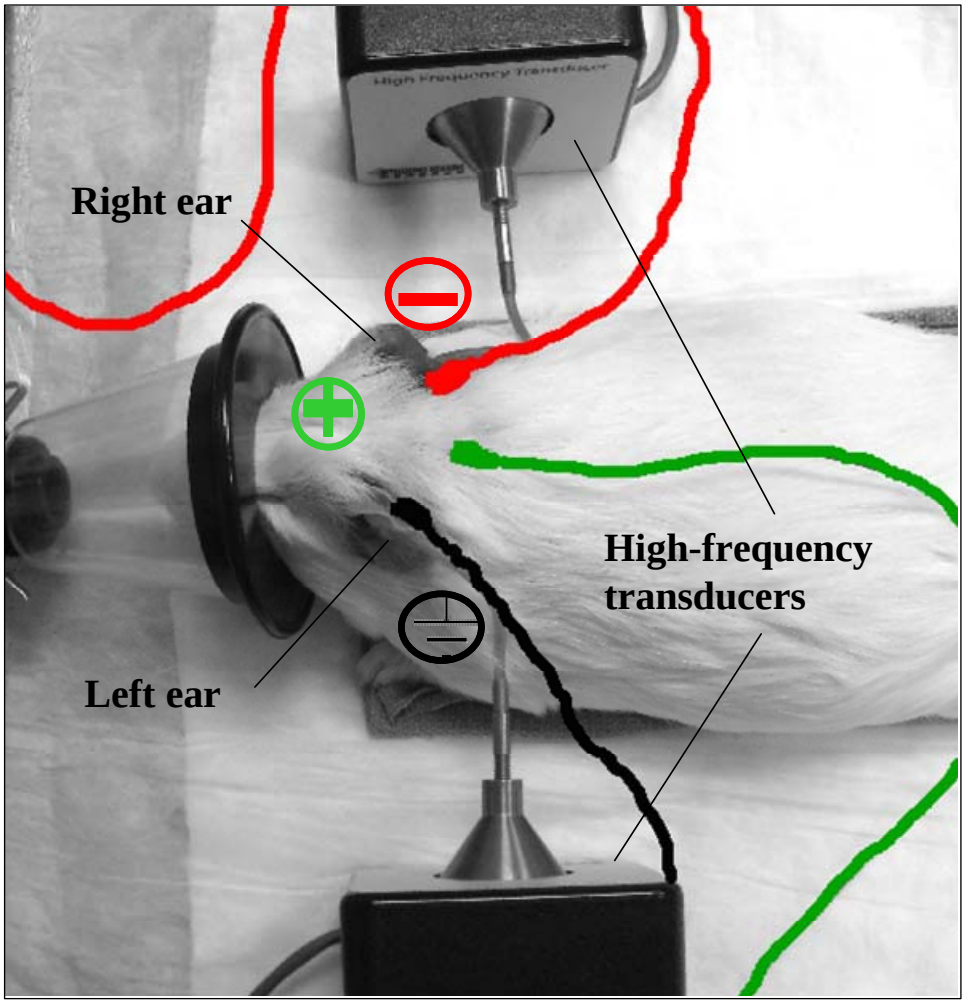

Figure 8. Enhanced photo of a guinea pig undergoing an ABR test for the right ear, displaying the position of the electrodes (left ear ground, vertex positive, right ear negative) and High-frequency transducers (McGill Auditory Sciences Laboratory 2009).

Chapter 14. Experimental Set-up

The study in the guinea pig model was categorized in three phases: establishing an optimal ototoxic dose of cisplatin, determining the effect of dexamethasone and determining the effect of dexamethasone on cisplatin ototoxicity.

Part I

To establish the optimal ototoxic dose of cisplatin in the guinea pig, 15 animals were divided into 3 groups. Each group was injected with 10 mg/kg (6 animals, n = 12), 12 mg/kg (3 animals, n = 6), or 14 mg/kg (6 animals, n = 12) of cisplatin. For groups testing cisplatin at 12 and 14 mg/kg, post-treatment ABR recordings were obtained on Day 3. For animals in the group treated with cisplatin at 10 mg/kg, in addition to Day 3 recordings, post-treatment ABR tests were obtained at one month after cisplatin, as well. The ears of all animals received no treatment. Pre-treatment baselines and post-treatment thresholds were compared to determine the threshold shifts.

Part II

Five control animals were used to determine the effect of IT dexamethasone on hearing thresholds. The experimental design was a bilateral study. Pre-treatment ABR thresholds were obtained for all animals. On the day before cisplatin administration, and on the day of cisplatin injection, both ears received a daily IT injection of either 4 mg/mL (2 animals, n = 4), or 24 mg/mL (3 animals, n = 6). Post-treatment ABR recordings were measured on Day 3, allowing time for any effusions to clear.

Part III

To determine the effect of dexamethasone on cisplatin ototoxicity, two different experimental set-ups were used: bilateral studies and unilateral studies.

For bilateral studies, pre-treatment ABR recordings were obtained in 12 animals. Six animals (n = 12) received IP cisplatin at 10 mg/kg, and six animals (n = 12)

received cisplatin at 12 mg/kg. On the day before cisplatin injection, and on the day of cisplatin injection, all of the subjects received IT dexamethasone (24 mg/mL) in both ears. If less than 0.01mL of dexamethasone was administered, a third application of dexamethasone was attempted on the day of cisplatin administration as well. Post-treatment ABR recordings were obtained on Day 3 for all animals, and additionally at Day 7 for animals treated with 10 mg/kg of cisplatin.

For unilateral studies, pre-treatment ABR tests were obtained in 26 animals. All animals received IP cisplatin at 12 mg/kg. On the day before cisplatin administration, and on the day after cisplatin administration, all animals received IT dexamethasone randomly in one ear (experimental ear), and sterile saline (0.9% NaCl) in their contralateral (control) ear. IT dexamethasone at concentrations of 4 mg/mL, 10 mg/mL and 40 mg/mL were administered to 6 animals (n = 6), 8 animals (n = 8), and 12 animals (n = 12), respectively. Post-treatment ABR recordings were obtained on Day 3.

Statistical Analysis

Mean ABR thresholds and mean ABR threshold shifts were averaged per frequency, or across all frequencies, and expressed as mean ± standard error of the mean (SEM). Comparisons of the mean ABR thresholds using a 2-tailed student's t-test were calculated to determine statistical significance. A p value of less than or equal to 0.05 was considered significant.

Chapter 15. Results

Part I

All animals treated with cisplatin experienced significant threshold shifts by Day 3 after cisplatin administration ($p < 0.01$). Figure 9 shows a dose-response curve comparing survival rate with the average threshold shift three days after cisplatin injection for all groups. In the group treated with 14 mg/kg of cisplatin, one ear was eliminated due to the death of the animal during ABR recording. For this group, the ABR threshold shift was 62.1 ± 8.5 dB. All animals treated at 12 mg/kg of cisplatin survived until Day 3 with a threshold shift of 57.2 ± 4.4 dB. For animals treated at 10 mg/kg, all survived until Day 3, and all but one animal survived until one month after cisplatin. At a dose of 10 mg/kg, the ABR threshold shift was 23.4 ± 6.1 dB at Day 3 ($p < 0.01$), and only 0.8 ± 3.5 dB after one month ($p = 0.68$). Average threshold shifts from baseline to Day 3 are demonstrated in Figure 10.

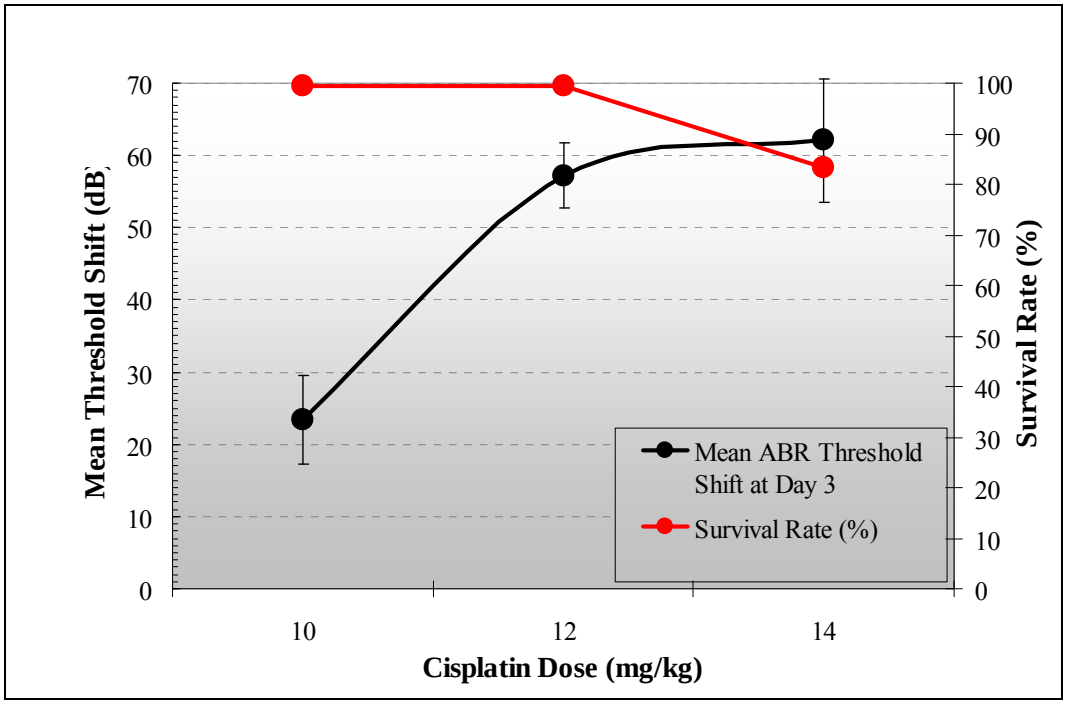

Figure 9. Mean ABR threshold shift across all frequencies and corresponding survival rate three days after intraperitoneal administration of cisplatin as a function of dosage.

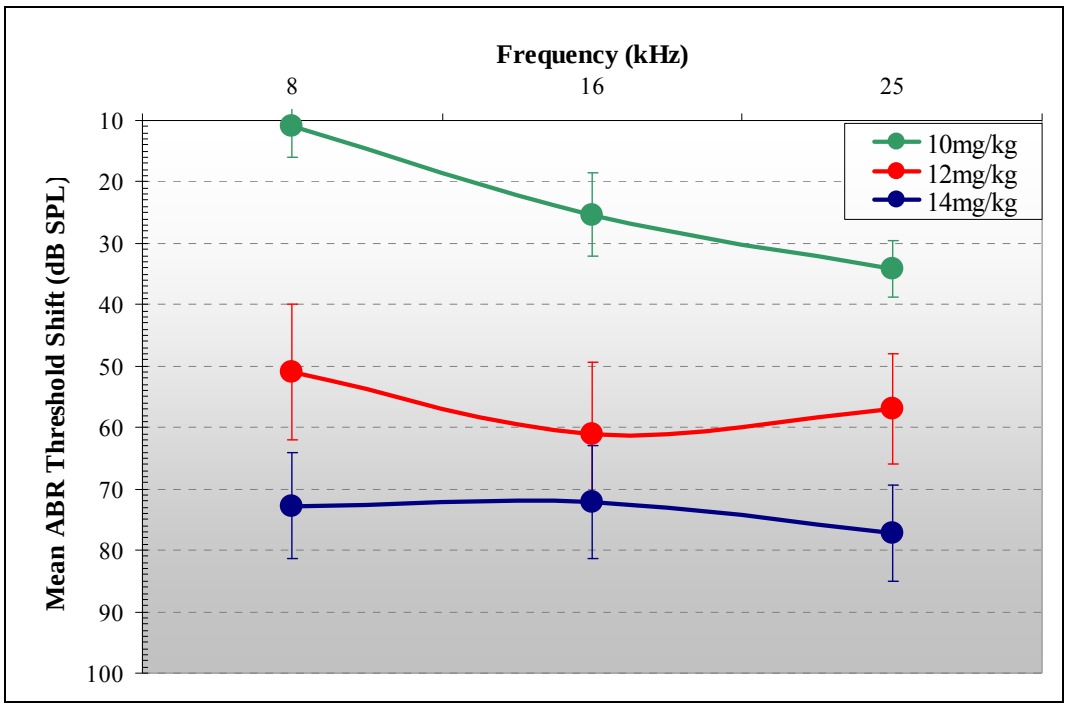

Figure 10. Mean ABR threshold shift per frequency for guinea pigs three days after treatment with 10 mg/kg (n = 12), 12 mg/kg (n = 6), or 14 mg/kg (n = 11) of intraperitoneal cisplatin.

Part II

Animals treated with IT dexamethasone at a low (4 mg/mL), or high (24 mg/mL) concentration experienced no significant hearing loss. In fact, animals treated with 4 mg/mL of dexamethasone showed a slight, but significant, improvement in hearing thresholds from baseline measurements ($p = 0.04$). Dexamethasone administered at 4 mg/mL and 24 mg/mL resulted in mean threshold shifts of -4.5 ± 3.4 dB and -2.7 ± 4.4 dB, respectively.

Part III

Bilateral Studies: For both groups of bilateral studies, all threshold shifts were significantly different from baseline recordings ($p < 0.01$). For animals treated with dexamethasone (24 mg/mL) and 10 mg/kg of IP cisplatin, three ears were eliminated due to infection. All animals in this group survived up to Day 7 recordings. Threshold shifts for animals on 10 mg/kg of cisplatin were 43.3 ± 8.0 dB at Day 3 and 46.1 ± 9.0 dB by Day 7. The lowest average threshold shift for this group (27.7 ± 7.2 dB) was observed at 8 kHz and the highest (55.5 ± 7.3 dB)

at 25 kHz. Threshold shifts for animals treated with 12 mg/kg IP cisplatin were more severe, at 52.7 ± 4.1 dB on Day 3. Average ABR thresholds for both groups are shown at baseline and at Day 3 in Figure 11.

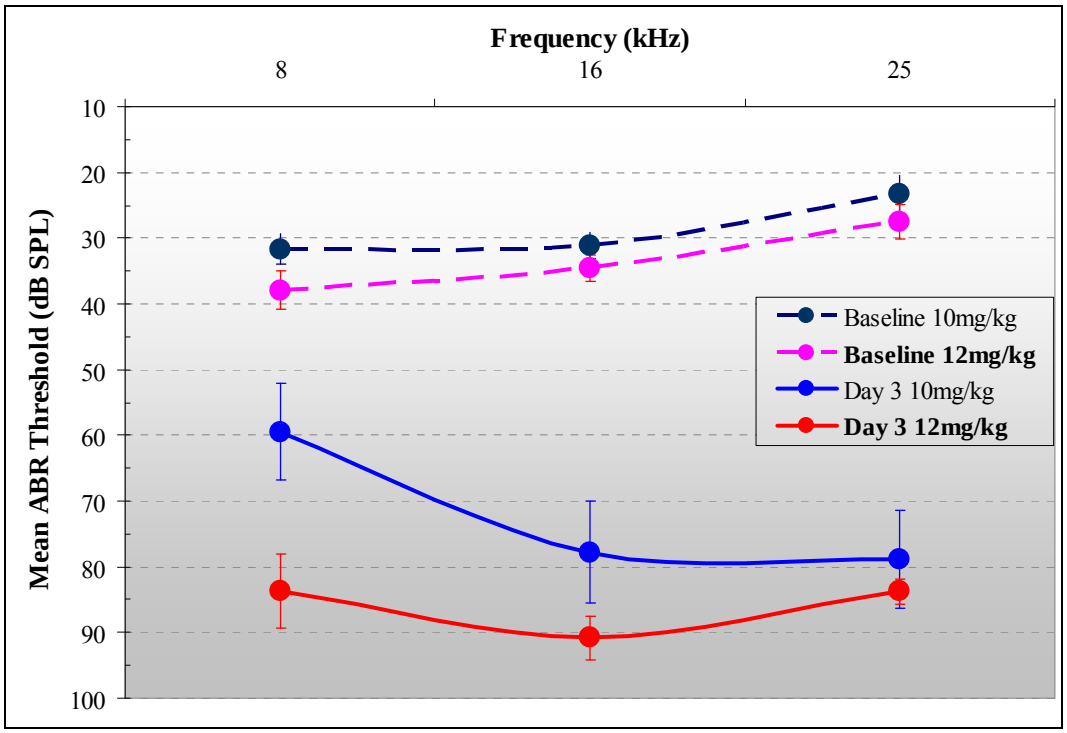

Figure 11. Mean ABR threshold per frequency three days after intraperitoneal cisplatin for animals treated with transtympanic dexamethasone (24 mg/mL) with cisplatin at either 10 mg/kg (n = 9), or 12 mg/kg (n = 12).

Unilateral Studies: For both saline and dexamethasone treated ears in all groups, there was a significant difference of threshold shifts from baseline ($p < 0.01$). A dose response related to the concentration of dexamethasone was not observed. Figures 12 and 13 demonstrate the average threshold shift for saline-treated and dexamethasone-treated ears. The greatest average threshold shifts of 57.7 ± 4.9 dB and 60.3 ± 3.7 dB were measured for control and experimental ears respectively in animals treated with 10 mg/mL of dexamethasone. The lowest average change in threshold (34.1 ±7.6 dB) was recorded at 8 kHz in experimental ears treated with IT dexamethasone at 40 mg/mL. Figure 12 displays average threshold shifts for control ears, labelled according to the contra lateral experimental ears. Figure 13 demonstrates the average threshold shifts for experimental ears.

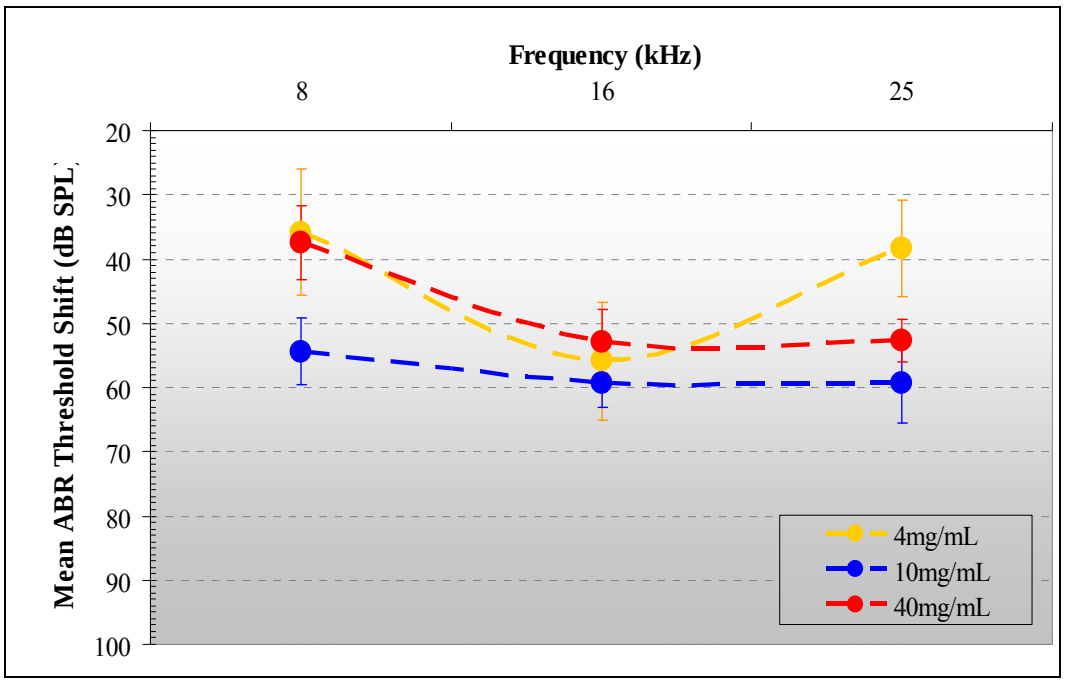

Figure 12. Mean ABR threshold shift three days after intraperitoneal cisplatin (12 mg/kg) for control ears treated with transtympanic saline (grouped according to the dexamethasone dose given to the contralateral ear; 4 (n = 6), 10 (n = 8), or 40 (n = 12) mg/ml).

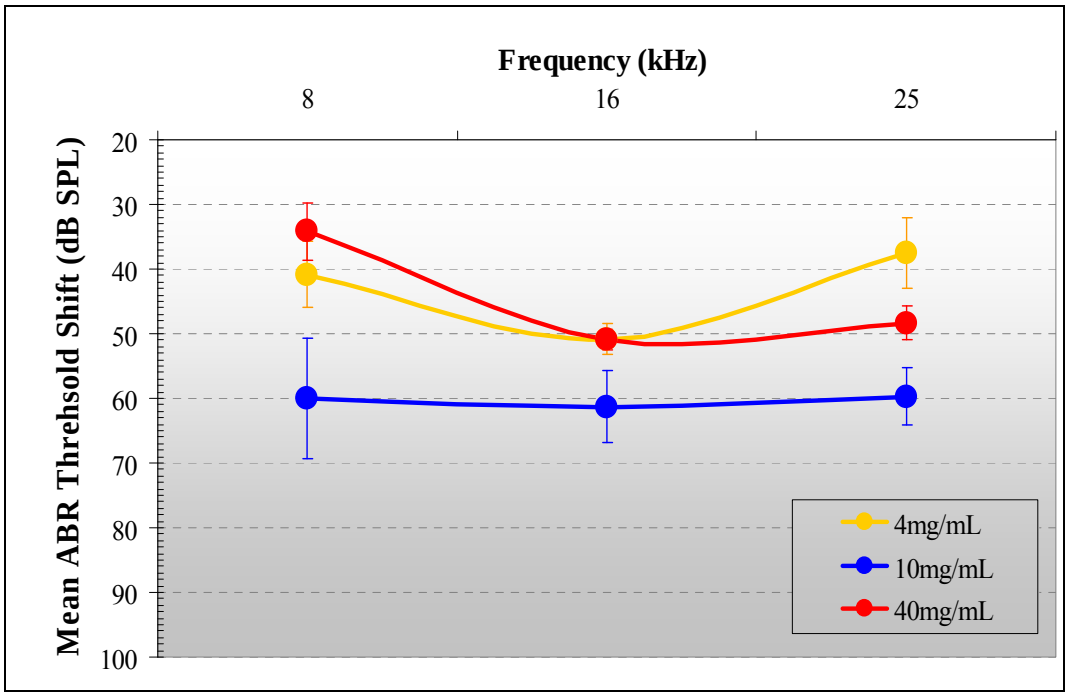

Figure 13. Mean ABR threshold shift three days after intraperitoneal cisplatin (12 mg/kg) for experimental ears treated with transtympanic dexamethasone at 4 (n = 6), 10 (n = 8), or 40 (n = 12) mg/ml.

Chapter 16. Discussion

Despite alterations in treatment, ototoxicity remains the leading, dose-limiting toxicity of cisplatin chemotherapy. Recent studies have measured the incidence of cisplatin ototoxicity as high as 88% in adults, with almost half of those patients requiring hearing aids after chemotherapy treatment [214]. Incidences are just as high in children, affecting well over half of those who receive cisplatin chemotherapy [74]. Especially in children, where hearing loss early in development can lead to numerous psychosocial difficulties, an effective treatment modality against cisplatin ototoxicity is urgently needed [215].

Over the years there have been many investigations into treatments against cisplatin ototoxicity. Antioxidants including many thiol-containing compounds such as D- or L- methionine, lipoic acid, N-acetylcysteine (NAC), glutathione ester and amifostine have been tested for efficiency against cisplatin ototoxicity in animal studies [6]. However, thiol-containing products have so far failed to offer protection in clinical trials [119]. There have been numerous animal studies into the use of non-thiol antioxidants as otoprotectants. For example, in addition to NAC, Choe *et al.* discovered a protective effect of Ringer's Lactate against cisplatin ototoxicity in the guinea pig animal model [123]. These studies have formed the basis for clinical studies into ototoxicity otoprotection that are currently ongoing [216].

This present study investigated the otoprotective effect of dexamethasone against cisplatin ototoxicity. This will constitute the first published study of high-frequency ABR measurements to test the effects of dexamethasone against cisplatin ototoxicity in the guinea pig model. This guinea pig study will also be the only published work to date testing for a dose response against cisplatin ototoxicity with dexamethasone. Although not statistically significant, results demonstrate that IT dexamethasone offered protection against cisplatin ototoxicity in the guinea pig model. This study extends the results of previous animal studies

into the otoprotective effects of IT dexamethasone. Daldal *et al.*, carried out studies in guinea pigs that demonstrated IT dexamethasone (4 mg/mL) protection of outer hair cell function up to 6 kHz, as measured by distortion product otoacoustic emissions [212]. Hill *et al.* investigated IT dexamethasone at 24 mg/ml in the murine model using high-frequency ABR testing and showed otoprotection at 8 and 16 kHz, but none at 32 kHz [211].

ABR thresholds, providing an accurate assessment of auditory function, including the capacity of the inner and outer hair cells, the spiral ganglion cells and neurons in the brainstem, were evaluated here. In addition, this study measured higher frequencies in the guinea pig, representing approximately 50% of the distance from the base of the cochlea, where cisplatin first exerts its damaging effects [217]. These results mirror those of Hill *et al.*, where protection was observed in the lower frequencies. Decreased protection of dexamethasone at the highest frequencies is expected, as the basal turn of the cochlea is most susceptible to cisplatin ototoxicity [211]. Damage of hair cells from cisplatin and other drug toxicities occurs in a gradient from base to apex. Lower levels of the natural antioxidant glutathione have been shown in the base of the cochlea, and may explain basal hair cell susceptibility to toxic damage by free radicals [218]. In this study, the damage to the basal turn by a large, single dose of cisplatin may have been too great to be prevented by IT dexamethasone. Lower, more progressive clinical schedules of cisplatin administration may result in less immediate and extensive damage to the basal hair cells resulting in a greater protection from IT steroids [211].

A clear dose response was not observed for unilateral studies with dexamethasone at varying concentrations. Dexamethasone at concentrations of 4 mg/mL showed a greater protection than IT dexamethasone at 10 mg/mL. For these concentrations, dexamethasone was obtained from commercially available IV solutions (Sandoz Canada). The dexamethasone at a concentration of 4 mg/mL contained a small amount of preservatives (methyparaben 0.15%, propylparaben

0.02%). It is unclear how this lowest concentration of dexamethasone could afford protection at the highest frequency tested. Dexamethasone at a 10 mg/mL contained a large amount of additives, including creatinine (8 mg/mL) and sodium citrate (10 mg/mL). Sodium citrate is a sodium salt of citric acid, a weak organic acid and natural preservative. Organic acids have been shown to cause ototoxicity in vitro, and most likely act to decrease the efficacy of dexamethasone here [219]. Dexamethasone used to create concentrations of 24 mg/mL (bilateral) and 40 mg/mL (unilateral) was obtained as a pure salt, with no added preservatives. It showed the greatest protection against cisplatin ototoxicity here, especially in the lower frequencies tested.

The otoprotective effect observed in the unilateral studies was also mirrored in the saline control ears. This phenomenon may be due to the effects of systemic absorption of dexamethasone from the experimental ear, exerting their effect in the control ears. However, in the study by Daldal *et al.*, the group treated with single dose cisplatin and bilateral IT saline (0.9% NaCl) only showed no significant differences ($p > 0.05$) in distortion product otoacoustic emission amplitudes between before and after treatment with cisplatin [212]. This and other studies indicate a trend towards otoprotection of normal saline itself, or other saline solutions such as Lactated Ringer's [123].

For bilateral experiments using IT dexamethasone at 24 mg/mL, greater protection was observed at the lower frequencies and with the lower dose of cisplatin at 10 mg/kg. The cumulative and dose-related effects of cisplatin ototoxicity are well documented [1-4]. A lower dose of cisplatin is expected to result in less damage to the cochlear sensory cells and a greater opportunity for protection from IT dexamethasone, as seen here.

IT steroids are currently used in the clinic as a safe and simple therapy for other cochlear disorders. IT injections result in fewer potential systemic side-effects, including negative interactions with cisplatin's antineoplastic effects, as well as

concentrating the drug where it is needed in the inner ear. Studies have shown that IT delivery of steroids results in much higher concentrations of the drug in the inner ear fluids when compared to parental dosing [220]. Therefore, IT dexamethasone in animal studies has excellent translational potential for immediate clinical use against cisplatin ototoxicity.

This study represents the first investigation into high-frequency otoprotection of dexamethasone in the guinea pig model, as well as the only animal study on the dose effect of IT dexamethasone. Guinea pigs have a well documented history for use in ototoxicity studies. Their range of hearing spans up to 50 kHz, which is slightly higher than, but still comparable to, a range of up to approximately 20 kHz in the human.

There are presently two main veins of cisplatin ototoxicity models: a single, large dose, and multiple, smaller doses over time [132, 212, 221-222]. A large, single dose model was used in this study due to its ease of implementation and management, and its proven efficacy as an ototoxicity model [221]. However, a more clinically-related, multiple dose regime could be implemented for future studies.

The present study measured ototoxicity for 3 days (72 hours) after cisplatin administration. It still remains to be determined whether this timeline is sufficient to show the full extent of cisplatin ototoxicity. For animal groups treated with cisplatin at 10 mg/kg only, there appeared to be a recovery of hearing after one month of cisplatin administration. An injection of 10 mg/kg can be considered a low dose for cisplatin-induced ototoxicity in the guinea pig. In this case, mechanical damage may have occurred, with minimal to no damage inflicted on the cochlear sensory cells which may have resulted in the recovery of hearing thresholds observed. (For example, conditions similar to TTS occurring with NIHL – See Ch. 4). Studies in rats suggest that hearing damage from cisplatin stabilizes after 5 to 7 days [223-224]. Single dose studies in the guinea pig and murine

models have measured the effects of cisplatin ototoxicity from 3 days, 8 days, or two weeks after cisplatin administration [211-212, 221].

The limitations of the current study include the small number of subjects per study group, the lack of inner ear sampling of dexamethasone and the lack of histological evaluations. Future studies should include cisplatin dosing to match clinical schedules, further investigation into the otoprotection of saline using bilateral control groups, testing of higher concentrations of dexamethasone, including ABR testing at lower frequencies, and histological comparisons.

Chapter 17. Conclusion and Summary

Cisplatin ototoxicity is a prevalent, irreversible and dose-limiting side-effect of cisplatin chemotherapy. With improved antineoplastic therapies, more patients are surviving their cancers to live with the toxic side-effects of cisplatin. Ototoxicity is common, and most worrisome in children, where hearing loss in the speech frequencies may have adverse psychosocial affects.

Antioxidants, such as thiol-containing compounds, have been investigated as possible otoprotectants against cisplatin ototoxicity, but have so far proven ineffective. Glucocorticoids, such as dexamethasone are powerful anti-inflammatories that have been shown to reduce the destructive free radical pathways of damage to the inner ear from cisplatin. In addition, dexamethasone has a well-established history of IT clinical use in the treatment of a variety of cochlear disorders.

In this study, a successful guinea pig model for cisplatin-induced ototoxicity is described. Through this model, IT administration of dexamethasone at varying concentrations was determined to have a protective effect on hearing thresholds. The effect was frequency and dose-related, with the most protection noted with the highest concentration of dexamethasone at the lowest frequencies tested. The safety of dexamethasone for IT use against cisplatin ototoxicity was also reaffirmed in the animal model.

Presently, there is no treatment for cisplatin ototoxicity. Here, dexamethasone presents as a safe, simple and useful treatment against ototoxicity in the guinea pig and shows excellent translational potential for clinical use in the fight against cisplatin ototoxicity.

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