Treatment of Cisplatin-Induced Sensorineural Hearing Loss Using Intra-Tympanic Dexamethasone: The Path of Implementation From Adults to Children

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To my father George and my mother Ghada who sacrificed beyond imagination for my success, thank you for teaching me, every minute, the true meaning of dedication and unconditional love.

To my fiancée and the woman of my life Farah, thank you for being the rock I lean on, the shield that reinforces me and the catalyst of my eternal youth.

"The feeling of awed wonder that science can give us is one of the highest experiences of which the human psyche is capable. It is a deep aesthetic passion to rank with the finest that music and poetry can deliver. It is truly one of the things that make life worth living and it does so, if anything, more effectively if it convinces us that the time we have for living is quite finite."

Richard Dawkins

Abstract

Background: Sensorineural hearing loss is the most apprehended adverse effect of platinum-based chemotherapy. This issue is particularly problematic in the pediatric population where hearing loss can have drastic repercussions on speech learning, language acquisition and social and interpersonal interactions of children. A number of recent studies have elucidated the molecular mechanism of platinum ototoxicity, highlighting the role of inflammatory mediators and free radicals responsible for the damage. Dexamethasone is one of most potent steroidal anti-inflammatory agents. It has been extensively used intratympanically to treat sensorineural hearing loss of different etiologies in adults, but has never been attempted in children.

Objectives: The objectives of this thesis are: (a) to review hearing physiology, sensorineural hearing loss, cisplatin ototoxicity, and dexamethasone. And (b) to evaluate if current evidence in the literature might justify a clinical trial of treatment and prevention of platinum-induced ototoxicity in children, by means of a systematic review and meta-analysis on the efficacy and safety of intra-tympanic dexamethasone in the adult population.

Methods: A comprehensive search of the literature yielded a number of randomized clinical trials on intratympanic dexamethasone treatment for sensorineural hearing loss. A systematic review and quality appraisal of these trials were conducted according to the Cochrane's collaborative tool for assessment of risk of bias, and a meta-analysis was carried out. A detailed examination of the side effect profile of the procedure was also undertaken.

Results: 12 randomized controlled clinical trials were included, 4 on Menière's Disease and 8 on Sudden Sensorineural Hearing Loss. Half of the studies had high risk of bias and a substantial heterogeneity was also noted. The meta-analysis failed to detect a statistically significant difference between intratympanic dexamethasone and alternative treatment (OR=0.39, 95% CrI= 0.13-1.07). The side effects profile was favorable for intratympanic dexamethasone. No serious adverse events were reported.

Conclusion: No evidence was found to support a difference between intratympanic dexamethasone and alternative therapies for sensorineural hearing loss. Larger trials are

recommended to determine the effectiveness of this treatment as compared to oral steroid therapy. It is recommended that there be a shift in study design selection towards non-inferiority or superiority studies. Avoiding systemic corticotherapy, especially in vulnerable populations, should be the rationale for future research in the field. The procedure can be labeled as safe, and its introduction in the pediatric population should be seriously considered.

Résumé

Introduction: La surdité de perception est l'effet indésirable le plus appréhendé de la chimiothérapie à base de platine. Cette question est particulièrement problématique chez les sujets pédiatriques où la perte auditive peut avoir des répercussions dramatiques sur l'apprentissage de la parole, l'acquisition du langage et les interactions sociales et interpersonnelles des enfants. Un certain nombre d'études récentes ont élucidé le mécanisme moléculaire de l'ototoxicité de platine, en soulignant le rôle des médiateurs inflammatoires et des radicaux libres responsables du dommage. La dexaméthasone est l'un des agents anti-inflammatoires stéroïdiens les plus puissants. Elle a été largement utilisé par voie intratympanique dans le traitement de la perte auditive neurosensorielle de différentes étiologies chez les adultes, mais n'a jamais été tentée chez les enfants.

Objectif: Les objectifs de cette thèse sont les suivants: (a) Examiner la physiologie auditive, la perte auditive neurosensorielle, l'ototoxicité induite par la cisplatine, et la dexaméthasone. Et (b) d'évaluer si les données actuelles dans la littérature médicale pourraient justifier une étude clinique randomisée sur le traitement et la prévention de l'ototoxicité induite par la cisplatine chez les enfants, à travers une revue systématique et une méta-analyse sur l'efficacité et la sécurité de la dexaméthasone intra-tympanique pour le traitement de surdité neurosensorielle dans la population adulte.

Le but de ce travail est de fournir au lecteur une revue de la physiologie auditive , la surdité neurosensorielle , l'ototoxicité induite par la cisplatine , ainsi que la dexaméthasone . En outre, nous présentons un nouvel examen systématique de la literature et une méta-analyse sur l' efficacité et l'innocuité de la dexaméthasone intra-tympanique chez les adultes , dans le but de générer des preuves scientifiques suffisantes qui alimenteront les recherches futures sur le traitement et la prévention de l'ototoxicité induite platine chez les enfants.

Méthodes: Une recherche approfondie de la littérature a abouti à un nombre d' essais cliniques randomisés sur le traitement de la perte auditive neurosensorielle par la dexaméthasone intratympanique. Un examen de qualité et une évaluation systématique de ces essais ont été menés selon l'outil collaboratif de Cochrane pour l'évaluation du risque de partialité des essais cliniques. Une méta -analyse a été réalisée en suite, en utilisant les outils d'analyse statistique

appropriés . Un examen détaillé du profil d'effets secondaires de la procédure a également été entrepris.

Résultats: 12 essais cliniques randomisés ont été inclus , 4 sur la maladie de Ménière et 8 sur la perte auditive neurosensorielle soudaine . 50% des études avaient un risque de biais élevé. Une hétérogénéité importante a été notée. La méta-analyse n'a pas détecté une différence statistiquement significative entre la dexaméthasone intratympanique et le traitement alternatif (OR = 0,39 , 95 % ICr = 0,13 à 1,07) . Le profil d'effets secondaires était favorable pour la dexaméthasone intratympanique. Aucun événement indésirable grave n'a été enregistré.

Conclusion: Aucune prevue n'a été trouvée pour établir une différence entre la dexaméthasone intratympanique et les thérapies alternatives pour la perte auditive neurosensorielle . De plus larges essais sont fortement recommendés pour déterminer l'efficacité de ce traitement par rapport à la corticothérapie systémique orale . De plus, un changement dans la sélection et la conception des études, vers des etudes de non - infériorité ou de supériorité, est fortement encouragé. Éviter la corticothérapie systémique, en particulier chez les patients vulnérables, devrait être la justification pour la recherche future dans le domaine . La procédure peut être étiqueté comme sûre, et son introduction chez les sujets pédiatriques devrait être sérieusement envisage.

Table of Contents

Preface

Contribution of authors

Dr. Nagi El Sabbagh was responsible for the literature review, the concept, design, data collection and analysis, as well as the drafting of the manuscript in chapter 3. Dr. Sam Daniel assisted in the conception of this manuscript. Data collection was done at McGill University. The literature review of the introduction (section 3.2), results interpretation (section 3.4), discussion (sections 3.5 and 3.6 and 4) and conclusion (section 5) were the work of Dr. El Sabbagh. Dr. Maida Sewitch provided expertise in the building of the study design, the interpretation of results and the drafting the second manuscripts. Dr. Lawrence Joseph conducted the statistical analysis of the second manuscript. Dr. Sam Daniel and Dr. Maida Sewitch provided supervision, guidance, clinical relevance expertise, and review of this thesis.

The design and conception of the original scope of this Master's thesis, which involved the bridging of investigation interest between the pediatric and the adult populations, was the work of Dr. El Sabbagh.

Claim of originality

The original idea of the need for a systematic review and a meta-analysis to assess the efficacy and safety of intratympanic dexamethasone injection for the treatment of sensorineural hearing loss in adults, before attempting it in the pediatric population was the conceived by Dr. Nagi El Sabbagh. The design of the meta-analysis in this thesis was envisioned by Dr. Nagi El Sabbagh and perfected by Dr. Maida Sewitch. No prior study in the medical literature has systematically reviewed and meta-analyzed all the randomized clinical trials on the efficacy and safety of intratympanic dexamethasone injections for the treatment of adult sensorineural hearing loss.

Acknowledgements

"The teacher who is indeed wise does not bid you to enter the house of his wisdom but rather leads you to the threshold of your mind". (Khalil Gibran)

First and foremost I would like to express my deepest gratitude to my supervisor Dr. Sam J. Daniel for his continuous guidance and relentless support over the last three years. His dedication and work ethics as well as his personal rapport make him a role model to future generations of researchers and physicians alike.

I would like to extend my most sincere feelings of gratefulness and appreciation for Dr. Maida Sewitch who stood by me through the hardest parts of my studies, who strengthened me with her wise advice, guided me patiently with her academic expertise and pulled me up at every pitfall along the road. Dr. Sewitch, you are a true inspiration.

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Working alongside these exceptional scientists and brilliant minds is a medal of honor that I will forever wear proudly.

1 Introduction

1.1 The incidence of platinum-induced hearing loss in the Canadian pediatric cancer population

The platinum-based chemotherapeutic agents cisplatin and carboplatin are two of the most widely used and successful chemotherapeutic agents. They are highly effective against a vast array of soft tissue neoplasms including cancers of the bone, brain and nervous tissue, as well as soft tissues of the head and neck among others (1,2). Cisplatin, and to a much lesser extent Carboplatin, have been long-term culprits in the development of platinum-induced ototoxicity in cancer patients (3,4,5,6). Ototoxicity is simply toxicity of the ear (oto- in Greek) that is the result of chemical insult from pharmacologically active molecules present in medications. Platinum-induced ototoxicity exclusively affects the cochlea, but less frequently the vestibular system, hence the focus of this thesis on sensorineural hearing loss. In their consensus review, Brock et al. estimated that, in general, the prevalence of platinum-induced ototoxicity in children is around 60% (7). In other studies published during the last decade, the incidence was found to vary between 60% and 90% (8); however, no such estimates exist in Canada. Due to advances in cancer screening and treatment protocols, up to 83% of Canadian children with cancer will survive for at least five years (9). This much welcomed survival rate comes at a hefty price: ototoxicity to which there is no cure or even prevention (10,11). Peleva et al. from the McGill Auditory Sciences Laboratory were the first to conduct a retrospective study to attempt to estimate the incidence of platinum-induced hearing loss in children from two major pediatric cancer centers in the Canadian province of Quebec. According to this study, 48% of the patients treated with platinum compounds suffered from post-chemotherapy ototoxicity, of which 30% was clinically significant. Among the patients with long-term follow up, 48% suffered from progressive deterioration of their hearing after completion of chemotherapy (12).

Until new studies shed light on this topic from a wider Canadian perspective, and with the standardization of nation-wide treatment protocols accounting for the variability in platinum dosage, these percentages can be considered representative of the Canadian pediatric population. A 2003 cost-benefit study in the United States projected the additional lifetime costs for one person with hearing loss to exceed 383,000 dollars (13), while Dionne and Rassekh et al. showed

that lowering the incidence of ototoxicity in Canadian children by 50% would lead to annual savings of almost 20 million dollars (14).

What is known so far about platinum-induced ototoxicity and its drastic impact on pediatric patients' quality of life, learning and social integration abilities, and socio-economic burdens it carries, has led many researchers to look deeper into novel therapeutic approaches to prevent and potentially treat this condition. A careful review of the literature for scientific evidence regarding Dexamethasone (DEX) used to prevent hearing loss in experimental animal models was conducted (15-19). In addition, a thorough review and assessment of the efficacy and the side effect profile of intra-tympanic DEX injections for the treatment of sensorineural hearing loss of various etiologies in adults were carried. The findings compelled me to intensify my search for a novel, concrete, feasible, accessible, cost effective and safe therapeutic approach for the prevention and treatment of platinum-induced ototoxicity in children.

1.2 Thesis rationale, objectives, and structure

Given the high incidence of profound and irreversible sensorineural hearing loss secondary to cisplatin ototoxicity, and its associated socio-economic burden, there is a strong need to find a prevention and even a cure for the morbidity that plagues pediatric cancer survivors and severely impairs their quality of life after completion of chemotherapy. However, a significant uncertainty remains about what is already known about this condition and how to achieve its prevention and treatment. This need and uncertainty formed the rationale of this thesis. As a result, the objective of this thesis is to examine the pathophysiology of platinum-induced hearing loss, to elucidate how the platinum chemotherapeutic agents exert their ototoxic effects on the molecular level, and to study the previously-reported therapeutic and side effects profiles of intratympanic Dexamethasone in treating sensorineural hearing loss in adults. The findings will help derive scientific recommendations for the potential implementation of intratympanic Dexamethasone in the prevention and treatment of cisplatin-induced hearing loss in the pediatric cancer population; something that has not been attempted to date.

Throughout this thesis, I sought to gather the necessary scientific knowledge to elucidate a novel approach to the prevention and treatment of platinum-induced ototoxicity in pediatric

cancer patients.

The thesis is structured as follows: It begins by describing briefly the anatomy of the target organ, the ear and namely the cochlea, and by explaining the basic physiological processes of the neural transmission of sound from its origin in the inner ear (section 2.1). Section 2.2 describes the 2 types of hearing loss in otology: Conductive Hearing Loss (CHL) and Sensorineural Hearing loss (SNHL), emphasizing the latter, which is the major concern in this thesis. The appreciation of the concepts of ototoxicity and hearing loss remains incomplete without an understanding of the audiologic evaluative techniques used for the screening, diagnosis and follow up of hearing status in the clinical practice and research alike. These techniques are overviewed in Section 2.2: The Pure Tone Audiometry (PTA), the Distortion Product Oto-Acoustic Emissions (DPOAE) and the Auditory Brainstem Response (ABR). In order to offer an objective and standardized assessment of ototoxicity, Section 2.2.4 describes the Chang Classification scale for the classification of hearing loss, that divides the different categories according to the frequency of sound affected in Hertz (Hz) and the depth or severity of hearing lost in decibels (dB).

Section 2.3 reviews platinum-based chemotherapeutic agents by first focusing on the culprit platinum-based chemotherapeutic drugs responsible for ototoxicity, and a brief examination of their clinical uses and indications, their systemic side effects and organ-specific toxicities, and the molecular mechanisms by which they exert their lethal effects at the level of the target end organ (section 2.3.3). Then Section 2.4 reviews usage of Glucocorticoids and Dexamethasone for prevention of Ototoxicity. Section 2.4.1 examines the characteristics of proposed therapeutic agents for the prevention of ototoxicity by reviewing the broad variety of their clinical uses and indications; Section 2.4.2 describes their well-known side effect profile; Section 2.4.3 highlights their applications in the prevention of platinum-induced ototoxicity in various animal models.

Chapter 3 presents a systematic review and a meta-analysis of all the randomized controlled clinical trials in the literature on "intra-tympanic injection of dexamethasone for the treatment of sensorineural hearing loss in adults". The results of this systematic review allow a better appreciation of the benefits and risks of intratympanic DEX prior to designing a study protocol for the administration of intra-tympanic DEX treatment in the pediatric population

suffering from platinum-induced ototoxicity.

Chapter 4 discusses the overall thesis. Chapter 5 concludes the thesis with a summary and a discussion of the study findings, and outlines the strengths and the limitations of the work, while highlighting the major implications of the findings on the clinical practice, especially on the future possibilities of introducing intratympanic DEX as a safe and efficacious treatment for ototoxicity in the pediatric population. The conclusion also presents the author's view of the future, through the pioneer idea of the "pan-Canadian randomized clinical trial" that the team led by Dr. Sam Daniel will implement. Finally, a new perspective is presented regarding the horizons and the potential benefits of the proposed treatment, at the level of patient-centered outcomes, as well as at the public health platform.

It is paramount to the reader's appreciation of this thesis to review in the next chapter, the basic hearing physiology, sensorineural hearing loss as well as the various diagnostic tools used to diagnose hearing loss. More importantly, it is essential for the reader to understand the mechanisms of action of cisplatin and how it exerts its ototoxic effect. Afterwards, the thesis examines dexamethasone as the most potent glucocorticoid, and elucidates its mode of action as an anti-inflammatory, and its role in the prevention and treatment of ototoxicity in animal models

2 Background & literature review

2.1 The ear, hearing and deafness

2.1.1 Basic Anatomy of the ear and physiology of hearing

The ear is divisible into three parts endowed with three distinct functions. Grossly speaking, the external ear serves as a funnel for the capture of sound waves in air. The middle ear amplifies and transmits the mechanical energy carried by the sound waves across two different media: from air to fluid. The inner ear transduces this mechanical energy, translates it into electrical impulses and transmits them to the auditory cortex for integration and interpretation. The external ear consists of the auricle or pinna, and the external acoustic meatus. The auricle serves to collect the vibrations of air particles by which sound is produced. The compressions of air particles travel at a speed of approximately 340 m/s and are conducted by the external

acoustic meatus to the middle ear. The middle ear is an irregular, laterally compressed space within the temporal bone, filled with air, and containing a chain of the movable bones: the malleus, the incus and the stapes which connect its lateral to its medial wall, and serve to transmit the vibrations from the tympanic membrane across the cavity into the internal ear. The footplate of the stapes is attached to the oval window at the base of the bony cochlea, and moves with every vibration transmitted from the tympanic membrane through the ossicular chain (20,21).

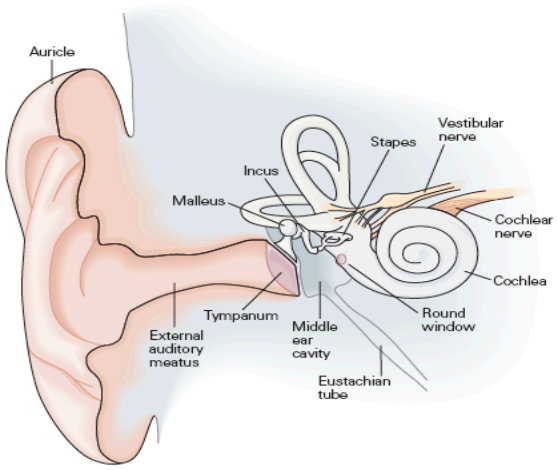

Figure 1 The structure of the human ear (Kandel principles of neuroscience)

The inner ear, or cochlea, forms the anterior part of the labyrinth and is the primary hearing organ. It is a spiral structure of progressively diminishing diameter wrapped like a snail's shell, in two and a half turns, around a conical bony core. The cochlea is roughly 9mm in diameter. It is covered with a thin layer of bone and embedded within the dense core of the temporal bone. The interior of the cochlea consists of three fluid-filled compartments named *scalae*: the scala vestibuli, the scala media and the scala tympani. In a cross section of the cochlea at any point along its course, the chamber farthest from the base is the *scala vestibuli,* at the basal end of which lies the oval window, the opening sealed by the footplate of the stapes acting as a piston as it pushes its fluid content with every air wave compression. The compartment closest to the cochlear base is the *scala tympani,* which also has a basal orifice, the round window, sealed by a thin elastic membrane. These two chambers are separated along their

course from base to apex by the cochlear partition, and only communicate with one another at the level of the helicotrema, where the duct tapers slightly below the apex. The fluid they contain is termed the perilymph. The third cavity is the scala media, which is located in the cochlear partition and is separated from the two other chambers by two elastic structures: Reissner's membrane separates the scala media from the scala vestibuli and the basilar membrane separates the scala media from the subjacent scala tympani; it is a complex structure involved in auditory transduction (22).

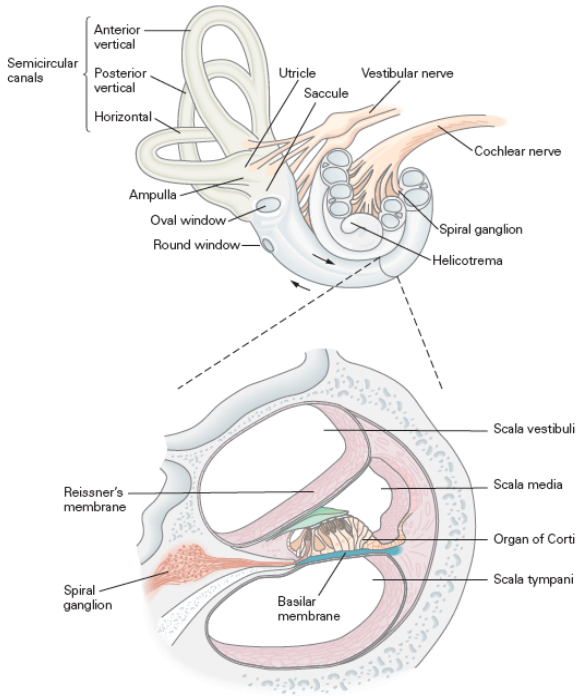

Figure 2 Cross section of the cochlea (Kandel Principles of Neuroscience)

Sound-induced increases and decreases in air pressure push and pull the tympanum, causing the ossicles to oscillate in a complex movement, according to the intensity and frequency of sound. The footplate of the stapes then acts as a piston, pushing against the perilymph of the scala vestibuli. The overall function of the middle ear is to ensure an efficient transfer of sound energy from the air outside the ear to the fluid of the cochlear compartments by matching the impedance between these two media. The uncompressible nature of the fluid in the scala vestibuli causes the pressure produced by the cyclic motion of the stapes to be dissipated downwards towards the elastic cochlear partition. The downward deflection of the cochlear

partition increases the pressure in the scala tympani and displaces its fluid and causing an outward bulging of the round window. Thus, each cycle of sound stimulation triggers a cycle of fluid oscillation in each of the three cochlear chambers. This up-and-down movement translates into corresponding movement on a more microscopic level, which eventually generates the neural signal from the organ of Corti (22).

2.1.2 the cochlea and the neural transmission of sound

The mechanical properties of the human basilar membrane are fundamental to our sense of hearing, as we know it as a species. In fact, microscopic studies have shown that the basilar membrane is thin and floppy at the apex, thick and stiff at the base, and about five times broader at the apex than it is at the base of the cochlea (22). This is inversely associated with a progressive increase in the cochlear chamber size from the apex to the base. Due to these mechanical properties, the basilar membrane does not respond uniformly along its length in response to different sound frequencies. It can roughly be compared to an array of strings ranging from the thickest bass cord to the thinnest “A” cord of a guitar. This characteristic is paramount in defining the tonotopic map of the basilar membrane and accurately predicts its reaction to different frequencies of sound stimuli. The relationship between sound frequency and position on the basilar membrane is logarithmic rather than linear, however, it transits smoothly. In fact, frequencies from 20Hz to 200Hz (180 Hz range), those from 200Hz to 2000Hz (1800Hz range), and those from 2000Hz to 20,000Hz (18,000Hz range), are each assigned approximately one third of the length of the basilar membrane. This spatial distribution of sound frequency responsiveness accounts for the human ear's impressive sensitivity to sounds that fall in the 2-5 KHz range. More so, sound is also decoded by intensity, which is translated into amplitude of the deflection wave along the basilar membrane. Therefore the basilar membrane acts as a mechanical frequency analyzer, by transmitting specific sound energies to underlying hair cells arranged along its length: this is the first step of frequency and intensity encoding in a sound, on its first step of its journey to the auditory cortex.

The organ of Corti is the receptor organ of the inner ear. It is an epithelial ridge that covers the basilar membrane. Each organ of Corti consists of roughly 16.000 hair cells aligned into three rows of Outer Hair Cells (OHCs) and a single row of Inner Hair Cells (IHCs) and

innervated by almost 30,000 afferent fibers that relay sensory information to the auditory cortex through the eighth cranial nerve. Correspondingly to the basilar membrane, the Organ of Corti and the nerve fibers are also tonotopically arranged so that at any segment, the hair cells are sensitive to unique frequencies, mapped logarithmically in ascending order from the apex to base of the cochlea. A second epithelial fold adjacent to the organ of Corti, but nearer the cochlea's central axis, gives rise to the tectorial membrane, a gelatinous layer that covers the organ of Corti. The tectorial membrane has a tapered distal end that forms a fragile connection with the organ of Corti (23). Upon vibration of the basilar membrane as a response to sound, the organ of Corti and the tectorial membrane undergo a similar motion. The different insertion points of the basilar and tectorial membranes causes a back-and-forth shearing motion of the upper surface of the organ of Corti and the lower surface of the tectorial membrane. This motion detected by hair cells causes a deflection of their tallest hair-like projection, the kinocilium, which opens some ion channels and closes others, modifying the permeability of the hair cell membrane in a complex physiological mechanism, producing a change in ion concentration across the membrane, creating an electrical gradient and triggering the neural signal through the afferent nerve fiber attached to the basilar surface of the hair cell (23). This is briefly, the complex mechanism of mechano-electrical transduction at the level of one hair cell. This phenomenon is responsible for the transformation of the mechanical energy of sound to an electrical energy in the form of a neural signal, ready to be integrated with thousands of others in the spiral ganglia of the cochlear nerve. At the level of the cochlear nerve, encoding of the stimulus intensity and frequency occurs, followed by a rapid transmission to higher centers of sound integration and eventually dispatched to the auditory cortex for interpretation.

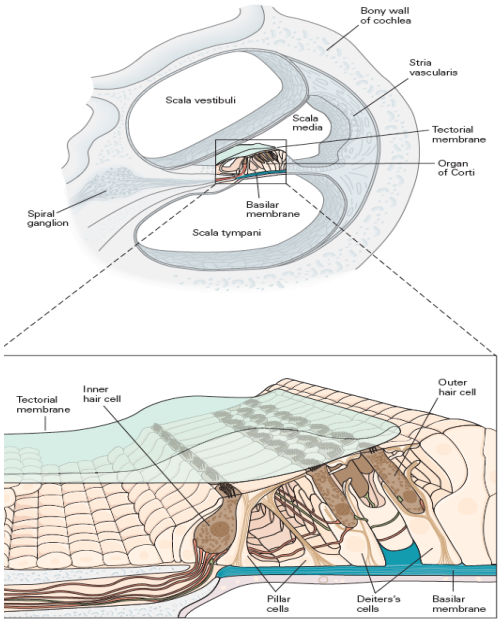

Figure 3 Cellular architecture of the human Organ of Corti (Kandel Principles of Neurocience)

2.1.3 Sensorineural hearing loss

Sensorineural hearing loss (SNHL) is a broad term that encompasses many etiologies of varying prevalence from infancy to childhood and adulthood. SNHL ranges from a partial decrease in hearing acuity to a total los of hearing in one or both ears, due to pathologies of the inner ear or along the auditory neural pathway, from the eighth cranial nerve, to the internal auditory canal and the brain. Otolaryngologists have tailored medical and surgical treatments to a number of pathologies that have been known to cause SNHL, however the majority of SNHL cases remain untreatable and disability is permanent. In the adult population, the most common cause of SNHL is presbycusis, an age-related condition that invariably affects men and women and is due to aging and degeneration of the inner ear hair cells. Other causes of SNHL include, but are not limited to, noise trauma, barotrauma, infections of the cochlear nerve, tumors such as acoustic neuromas, autoimmune and metabolic disorders, Menière's disease. One major contributor to acquired SNHL in the adult population is systemic exposure to ototoxic agents including the antibacterial family of the aminoglycosides, aspirin, and chemotherapeutic platinum-based agents (24). The platinum-based chemotherapeutic agents cisplatin and carboplatin are the focus in this thesis because of the potential preventability and reversibility of their ototoxic effects.

The epidemiology of SNHL in children is different from that in adults and carries with it serious implications for acquisition of speech and the adequate development of social and communicative skills. Prematurity and congenital etiologies represent the bulk of the disease burden and account for the wide heterogeneity in the nature of the offending agent. Infant graduates of the neonatal intensive care units have a substantially higher risk of SNHL secondary to perinatal hypoxia or anoxia (25). Intrauterine infections account for a significant proportion of neonatal SNHL and the main culprit is cytomegalovirus, followed by Toxoplasma gondii (congenital toxoplasmosis), Treponema pallidum (Congenital Syphilis), Herpes Simplex Virus 1 and 2 (neonatal Herpes Simplex meningoencephalitis) and Rubella (24). In the perinatal period, Group B streptococcal (GBS) infection is a major cause of sepsis, whose survivors often exhibit sequelae of SNHL (26). Later during childhood, measles and mumps infections have been associated with SNHL complications (27). Furthermore, a study by Brookhouser and colleagues revealed that 15 to 20 percent of children with bacterial meningitis develop profound bilateral and irreversible SNHL (28), whereas Richardson and colleagues concluded that meningitis-associated hearing loss occurs during the first hours of the disease and does not appear to be ameliorated by any antibacterial regimen (29). On the other hand, hereditary (genetic) bilateral SNHL occurs in about 1 in 2000 births and accounts for up to 50% of childhood SNHL (30), of which 80% of cases are inherited in an autosomal recessive pattern, 15% are autosomal dominant, 2% are X-linked recessive and 1% mitochondrial (31). Hereditary SNHL can be full blown at birth, progressive from birth, or it may develop when the child is older. Such include rare congenital malformations of the temporal bone and the inner ear such as the Michel, Mondini, Scheibe and Alexander malformations as well as perilymph fistulas and many other rare syndromes.

This thesis will focus on SNHL caused by the platinum-based chemotherapeutic compounds in children. The thesis will analyze and criticize what has been accomplished so far in this field in adults, and try to apply it into the pediatric population. The main reasons to study this pathology are 1) the abundant amount of data and studies produced by our auditory sciences laboratory at McGill and 2) the potential preventability and reversibility of this condition. The diagnostic modalities, their clinical applications and the quantification and classification of hearing loss are presented below.

2.2 The Audiologic evaluations of sensorineural hearing loss and the standard assessment of ototoxicity

2.2.1 The Pure Tone Audiometry (PTA)

Puretone thresholds are the lowest level of tonal stimulus amplitude to which a person responds (32). Puretones are defined by a set of characteristics: phase, duration, and most importantly frequency and amplitude. Humans are able to perceive sounds in the frequency range of 20-20,00Hz, but the human ear has evolved to be mostly sensitive to sound frequencies in the range of 250-8,000Hz, which encompasses those frequencies important in speech recognition and understanding (33). Puretone amplitude is quantified in decibels (dB) of Hearing Level (HL), which the logarithmic scale most widely used in the clinical setting. Puretone thresholds provide quantification of the degree of hearing loss resulting from outer and middle ear pathologies separately from those arising from of the cochlear or auditory nerve pathologies. This ability to distinguish, conductive from sensorineural hearing loss, makes the puretone thresholds a very important means, and one of the universally used initial tests in the establishment of a diagnosis and the guidance and implementation of treatment strategies. Audiometers quantitatively assess both bone and air conduction of sound in each ear separately. The detailed description of how and audiometer works lies beyond the scope of this thesis, but it remains important to clarify that air conduction is assessed using earphones whereas bone conduction uses vibrators on the patient's skull in order to bypass the outer ear. As a general rule in interpreting audiograms, when both AC and BC hearing thresholds are elevated, the pathology is most likely to be cochlear or neural in origin and thus "sensorineural". However if thresholds are poorer by AC than by BC, the source of the hearing loss is at least partially related to the outer or the middle ear (32). Since it is administered by audiologists, and its data is collected from patient feedback (clicking when they hear the sound), the audiogram has a few limitations that pertain to human error, such as the test-retest reliability, the test's unfeasibility non reliable patients namely those with severe cognitive impairment that precludes understanding and following directives, and children less than 5 years old. Another constraint is seen in patients suffering from tinnitus, which causes an increase in the false positive clicks during the test and therefore help skew the audiogram towards underestimating the degree of hearing loss. Nonetheless, the Pure Tone Audiometry remains the number one, first line diagnostic tool for the screening, diagnosis and follow-up of hearing status in almost all patients.

Over time, other modalities of hearing assessment were developed to complement and often compensate for the limitations and shortcomings of the Pure Tone Audiometry. In the following sections we discuss the Distortion Product Oto-Acoustic Emissions (DPOAE) and the Auditory Brainstem Response (ABR). As we finalize this section, we compare, contrast and appreciate how these hearing assessment tools can synergistically provide the otolaryngologist with complementary and crucial data to form a diagnosis of the underlying condition.

2.2.2 The Auditory Brainstem Response (ABR)

Unfortunately, the puretone audiogram does not always provide accurate and reliable information about the patient's hearing threshold. This is particularly true in the case of infants, toddlers, and newborns and, as mentioned earlier, patients with severe cognitive or motor impairment, as well as those who intentionally falsify their response. For this wide patient population the physician needs a more "objective" measurement tool that does not require active cooperation, task comprehension and behavioral response: The Auditory Brainstem Response.

After an initial audiogram, the physician can usually evaluate the degree of hearing loss and estimate its nature: Conductive (outer or middle ear origin), or Sensorineural (Inner ear, cochlear nerve or central auditory pathways). Nevertheless, in a large number of SNHL cases, the exact location of the lesion is often difficult to ascertain even with the help of modern technological imaging modalities. The ABR is a neurodiagnostic tool developed to aid in the differential diagnosis of lesions of the cochlea (namely Menière's disease), the cochlear nerve and the brainstem.

The ABR is composed of voltage deflections occurring within the first 15 milliseconds after the onset of an auditory stimulus. These peaks and troughs in voltage represent the activity of downstream neural elements along the auditory pathway: the eighth nerve and the brainstem. (34). The major determinants of an ABR are the latency and amplitude of its peaks. As tumors of the auditory pathway exert pressure on the nerve fibers of the cochlear nerve or the brainstem, they cause a delay in the transmission of neural activity, resulting in longer peak latencies of the activity of these fibers. Early studies on the ABR reported sensitivity in the range of 95% to 98% (35).

Despite its wide use in specific patient populations, as well as its status as first line hearing screening modality in newborns, the ABR has limitations. Eggermont and colleagues (39) concluded that ABR methodology fails to detect tumors smaller that 1.0cm in size. This conclusion was later supported by studies comparing the sensitivity of ABR to Gadolinium-enhanced Magnetic Resonance Imaging (40-49). In fact, these studies found that ABR latency measures detected all intracanalicular and extracanalicular tumors larger than 1.0cm. The sensitivity of the ABR latency measures across these studies varied between 63% and 93% with false negative rates in the 7%-37% range (50,51). This led to the overall conclusion that Gadolinium-enhanced MRI is the gold standard for the detection of eighth cranial nerve tumors as small as 3.0 mm. Nevertheless, the ABR studies remain the initial tool in the initial workup of a suspected eighth nerve tumor.

2.2.3 The Distortion Product Oto-Acoustic Emissions (DPOAE)

Otoacoustic Emissions (OAEs) are a fascinating auditory phenomenon. They are sounds that originate in the cochlea and follow a retrograde route, to the middle and outer ears and can be measured using an ultra sensitive microphone. Since their discovery by David Kemp in 1978 they have been the subjects of intensive research that examined their relationship to auditory function.

OAEs are preneural emissions, which means they are still produced even when the eighth cranial nerve has been transected (52). Unlike neural responses, OAEs are unaffected by stimulus rate or amplitude (53,54), however they are vulnerable to acoustic trauma (55), hypoxia (56) and ototoxic medications (57,58), which cause hearing loss secondary to OHC damage. In 2004, Liberman et al demonstrated that OHC contribute significantly to the "cochlear amplifier" by virtue of their somatic mobility as well as the motion of their stereocilia (59). In 1999 Knight and Kemp established that OAEs measured in the ear canal are likely a combination of energy from two mechanisms: "non-linear distortion" and "linear coherent reflections" (60).

OAEs are traditionally classified into spontaneous and evoked. DPOAEs are of the "evoked" type. They are measured by subjecting the ear to 2 puretone stimuli, called "primers", that have different frequencies such as f1< f2, and different levels L1 and L2. When the 2 frequencies are reasonably close they hit the cochlear basilar membrane and two very closely

adjacent sites, resulting in an output of energy from the cochlea at other discrete frequencies that are arithmetically related to the frequencies of the two original primaries. “Distortion Product” OAEs can therefore be measured using narrowband filtering at the frequency of interest. The 2f1-f2 DOPAE has the largest level in human ears as compared to other DOPAEs and is thus the most widely used DPOAE in the clinical setting (61).

After reviewing in considerable details the various hearing assessment methods and their advantages and disadvantages, we introduce in the next section a classification specifically designed, validated and implemented for the measurement of cisplatin-induced hearing loss: The Chang Classification.

2.2.4 The Chang Classification for hearing loss

In clinical practice, it is recommended to screen for baseline hearing level or hearing loss in all patients receiving ototoxic medications, prior to initiation of therapy, prior to successive doses and if symptomatic hearing loss is clinically suspected (62). A number of hearing loss grading scales have been developed since the early days of otolaryngology. None of them dealt specifically with hearing loss secondary to ototoxicity, and almost all of them were used inconsistently in research, and when translated into the clinical setting, a large number of discrepancies and variability of results were noticed. A pioneering work by Chang and Chinosornvatana in 2010 provided the medical community with the first practical classification for the evaluation of cisplatin-induced hearing loss in the pediatric population (62). The ingenuity in this article lies in the manipulation and re-adaptation of the very widely used “Brock criteria” to include the clinically important frequency of 8 KHz and exclude the less outcome-significant 3 and 6 KHz frequencies which detected more clinically significant hearing loss. Modification of the Brock criteria yielded a more clinically robust grading system: “The Chang Classification”, shown in the table below.

Chang Grade	Sensorineural Hearing Loss Threshold (dB HL) Bone conduction or air conduction with normal tympanogram
0	≤ 20 dB at 1, 2 and 4 KHz
1a	≥ 40 dB at any frequency 6 to 12 Hz
1b	> 20 and <40 dB at 4 KHz
2a	≥ 40 dB at 4 KHz and above
2b	>20 and <40 dB at any frequency below 4 KHz
3	≥ 40 dB at 2 or 3 KHz and above
4	≥ 40 dB at 1 KHz and above

Table 2.1 The Chang Classification. Source: Chang KW, Chinosornvatana N. Practical grading system for evaluating cisplatin ototoxicity in children. Journal of clinical oncology: Official journal of the American Society of Clinical Oncology. Apr 1, 2010;28(10):1788-179.

As a result, the Chang grading system was found to be a clinically important tool of ototoxicity measure. There was a strong correlation between the grade and the audiologist's recommendation for hearing aid (62). Another strength of this grading system is its correspondence with the actual pathophysiology of ototoxicity (starting at high frequency and progressing to the lower frequencies) (62). Also, it has a logical progression of increasing clinical impact with increasing grade. Furthermore this grading system facilitates the communication between the healthcare professionals implicated in patient care, by simplifying the language of communication between audiologists, otolaryngologists and oncologists, using a common language denominator (grade 1a, 1b, 2a, 2b 3 and 4 ototoxicity instead of the convoluted audiologic descriptions of mildly or severely down-sloping mild-to-moderate sensorineural hearing loss). Finally, this grading system is tailored to account for hearing loss impact on speech, language and need for assistive hearing devices, which makes it a patient-centered grading system that interprets the severity of the audiologic hearing loss in light of its impact on the patient's quality of life.

In conclusion, since this grading system specifically deals with cisplatin-induced ototoxicity, it was found to be the most valuable hearing loss assessment tool for use in future pediatric cisplatin-ototoxicity prevention clinical trials.

2.3 The platinum-based chemotherapeutic agents

2.3.1 Overview, therapeutic uses and clinical indications

Cis-diamminedichloroplatinum (II) also known as cisplatin (CDDP) was discovered in 1965 when Rosenberg and colleagues experimented platinum electrodes on Escherichia coli cultures, which resulted in inhibition of cell division (63). Since then, research intensified to elucidate the mechanism of action of this compound and translate it into cancer treatment. Cisplatin toxicity is non-cell cycle specific. In fact, cisplatin exerts its effects by covalently binding purine DNA bases, forming both intra and inter-strand crosslinks that become impenetrable by the nuclear enzymes that govern DNA replication. Despite the cell's auto-repair mechanisms, the damage inflicted on the DNA molecule results in the inevitable event of cell death by means of apoptotic and non-apoptotic pathways (64). 90% of cisplatin in the plasma is protein bound and reaches its peak concentration 1-hour post intravenous administration (65).

Cisplatin has a half-life of 24hrs at therapeutic doses (66), and around 28 days at toxic doses (67,68). It does not permeate the blood brain barrier (69) and concentrates mainly in the kidneys and liver, which are its sites of metabolism and excretion at approximately 90% and 10% respectively (70,71).

Cisplatin is the cornerstone of the treatment of many soft tissue malignancies, namely ovarian, testicular, esophageal, head and neck, bladder, gastric and lung cancers. Since its introduction as one of the main solid organ antineoplastic agents, it has dramatically improved the survival of cancer patients, both in the pediatric and adult populations. The standard dose range in adults with normal kidney function is 50-100 mg/m^2 per cycle, given at 3 to 4 weeks intervals. With the development of pretreatment hydration protocols for nephroprotection, neurotoxicity became the major dose-limiting factor (65).

The major route of cisplatin administration is intravenous. Intraperitoneal infusion has been attempted for ovarian cancer, but the severe toxicities that ensued precluded its routine administration via the peritoneal cavity (78). The intra-arterial route has been successfully used in selected cases of head and neck cancers, in which cisplatin acted mainly as a radio-sensitizer prior to radiotherapy.

Tumor cells have developed different mechanisms to evade or resist cisplatin's toxic effects. Four mechanisms of resistance have been described to date. These include:

1) Intracytoplasmic deactivation through binding to peptides and proteins such as glutathione and metallothionein (72-75).

2) DNA repair through the Nucleotide Excision Repair (NER) system of the host cell (76,77).

3) Modification of cellular accumulation by means of active efflux (78).

4) Alteration of the cell's apoptotic pathway by mutation to the mismatch repair genes (MMR), which modifies the cell's sensitivity to cisplatin (78).

As a very potent and efficacious antineoplastic agent, cisplatin has earned its status as one of the most successful drugs for the treatment of a wide array of solid and hematological tumors. In select cases of head and neck cancers non-amenable to surgery, cisplatin has even been used as a single agent in a monotherapy regimen and showed significant efficacy in controlling the underlying malignancy.

Despite the widely acclaimed success of cisplatin, its toxicities and side effects often act as limiting factors and force the healthcare provider to alter the dose administered, integrate other drugs into the regimen and even switch to a completely different family of antineoplastic agents. In the coming section, we review the most common and severe toxicities and side effects of this drug.

2.3.2 Systemic side effects and toxicities

Given its non-specific targeting, cisplatin often causes adverse effects affecting the bone marrow, the kidneys, the peripheral nervous system as well as the gastrointestinal tract (65). The incidence and magnitude of these adverse events are dose-dependent. However, toxicities are not an exclusive characteristic of overdosing, and have been reported in the literature even at therapeutic and low doses. This is particularly true, when other factors come into play, such as the patient's age, weight, comorbidities, baseline renal function and concomitant use of other nephrotoxic medications. To date, there are no guidelines to address the management of cisplatin toxicity. All clinical efforts to reduce the systemic impact of this drug on the vital organs have barely scratched the surface of the problem, and have revolved around hydration for the purpose of renal protection.

Immediate cisplatin toxicity is non-dose-dependent. It includes acute nausea and vomiting which occur within a few hours after intravenous administration despite the implementation of routine antiemetic prophylaxis (80-83). Delayed nausea and vomiting occur more that 24 hours after cisplatin administration and can last for up to two weeks. These are usually observed in high doses and overdoses of cisplatin. Expectedly, the combination of cisplatin with other antitumor drugs usually potentiates the gastrointestinal toxicities (65).

Nephrotoxicity is also dose-dependent. Acute and chronic renal failure are common adverse effects of cisplatin therapy, the mechanisms of which have not been appropriately elucidated (84-85). Cisplatin-induced acute tubular necrosis and the resulting renal electrolyte wasting contribute to the development of metabolic acidosis and life-threatening electrolyte imbalances including hypomagnesemia (86), hypocalcemia (87), hypophosphatemia, hypokalemia (88), hyponatremia (89) and hyperuricemia.

2.3.3 Cisplatin-induced ototoxicity: the molecular mechanisms and clinical manifestations in children

The effects of platinum-based chemotherapeutic agents on normal tissue cells including inner ear cells are presumably not different from those on neoplastic cells. As described in the introduction, ototoxicity, the price that cancer children end up paying for the platinum chemotherapy, is laden with serious morbidity and decline in quality of life. How does ototoxicity occur? What are the molecular events that lead to inner ear cell death, and what are the clinical manifestations in children? Uptake from the stria vascularis into the endolymph, and entry into the apical membranes of hair cells, appears to be the first event in the sequence leading to cell damage (7). After uptake, several mechanisms seem to be implicated in cisplatin-induced ototoxicity, and are highlighted in the medical literature. One mechanism describes binding of cisplatin to the guanine base in the cell's DNA, followed by inter and intra-strand crosslinks, activation of the p53 gene, arrest of the cell cycle and culminating in apoptosis (90). Casares et al recently described another mechanism consisting of generation of free radicals and reactive oxygen species that lead to lipid peroxidation, alteration of enzymatic structure and function leading to apoptosis (91). After binding to the cochlear cell's DNA, cisplatin induces dysfunction in the antioxidant enzymes glutathione reductase, glutathione peroxidase, superoxide dismutase

and catalase on one hand, and saturates them with free radicals on the other hand, overwhelming therefore the antioxidant apparatus of the cochlear cell (92). Since the cochlea is essentially anatomically isolated, it functions mostly as a closed system and thus cannot excrete toxins at the same rate they are generated in the presence of a defective and saturated intracellular antioxidant mechanism. Free radicals end up accumulating in the hair cells, supporting cells, stria vascularis and the auditory nerve, leading to their eventual apoptosis (93). Another complementary mechanism of damage has been recently proposed and validated: the inflammatory pathway. Kim et al. have clearly demonstrated the roles of pro-inflammatory cytokines, including tumor necrosis factor, Interleukin-1β, Interleukin-6, and subsequent reactive oxygen species (ROS) generation on the pathogenesis of cisplatin ototoxicity in vitro and in vivo (94). It has been recently shown that STAT1 and STAT 6 signaling pathways play a pivotal role in cisplatin-mediated pro-inflammatory cytokine production and ototoxicity (94). For instance, STAT1 siRNA protected against activation of p53, reduced apoptosis, reduced damage to outer hair cells and preserved hearing in rats. STAT1 siRNA also attenuated the increase in inflammatory mediators, such as TNF-α, which protected cells from cisplatin-mediated apoptosis (95).

As stated in chapter one, the reported rates of cisplatin-induced ototoxicity in the pediatric population are highly variable, ranging from 60 to 90% across the literature (8). This variability is due, in part, to the differences in cisplatin treatment protocols, and in another part to the genetic polymorphism that dictates the individual's susceptibility to ototoxicity. Moreover, the absence of a unified standardized ototoxicity grading scale and the significant time-window variability in assessing ototoxicity make the comparisons more difficult.

Cisplatin ototoxicity usually manifests as bilateral, symmetrical, irreversible sensorineural hearing loss (96), often associated with tinnitus and vertigo (97), beginning at high frequencies and progressing to the speech frequencies namely those below 4 kHz. This high frequency hearing loss may compromise speech recognition and comprehension in young children by rendering certain consonants inaudible (98). Furthermore, it impairs musical and other-environmental-noises perception, resulting in a poorer quality of life (99). Given that adequate hearing is a prerequisite for speech acquisition, young children affected by cisplatin ototoxicity are at a higher risk of neurocognitive deficit and psychosocial retardation (100). Oftentimes, hearing loss continues to worsen even after completion of cisplatin therapy (98),

which puts pediatric cancer survivors at risk of learning impairment, school performance retardation, and social interactions (101-102). Even if hearing loss is mild, children have been found to suffer from poor word recognition and analysis, impaired phonological short-term memory and discrimination, as well as poor reading skills (103). Moreover, development of ototoxicity may hinder cancer treatment as, once ototoxicity occurs, the dosage of cisplatin has to be significantly reduced and the choice of chemotherapeutic agent has to be reconsidered to prevent further hearing loss (104). Since cisplatin begins by affecting the high frequency ranges first, early stages of ototoxicity are often asymptomatic and can easily be missed on routine audiometry (98). Extended High Frequency audiometry allows for an earlier detection of ototoxicity well before it becomes clinically evident in otherwise asymptomatic children (105).

Some culprit risk factors have been associated with increased risk of cisplatin ototoxicity. Cisplatin loading dose, dose per course and overall cumulative dose have been identified as the major determinants of cisplatin ototoxicity (100,106). In addition to cisplatin dosage, concomitant treatment with ototoxic drugs such as aminoglycosides or furosemide has been found to potentiate cisplatin-induced hearing loss. Furthermore, Children less than 5 years old (106) and those who received cranial radiation therapy as part of their cancer treatment (107) are at an increased risk of developing cisplatin-induced hearing loss.

In the next section, Dexamethasone, the most potent of glucocorticoids, will be overviewed, in an attempt to elucidate its molecular activity especially in halting the inflammatory intracellular processes. Has Dexamethasone's otoprotectant effect been studied in animals? Has it ever been used for the treatment of sensorineural hearing loss in adults? The answers to these questions will help open a crack in the wall that separates us from better understanding cisplatin-induced ototoxicity prevention.

2.4 Glucocorticoids and Dexamethasone

2.4.1 General overview

In the 1950s, the chemical modification of endogenous glucocorticoids opened the door to an era of novel therapeutics for a wide variety of inflammatory and immunologic diseases. For instance, prednisolone, a synthetic glucocorticoid that is four times more potent than the naturally occurring cortisol, was created by adding a double covalent bond between the first and

second carbon atoms of cortisol (108, 109).

Most of the circulating cortisol is bound to corticosteroid-binding globulin and albumin. A large portion of the biologically available cortisol can also be bound to red blood cells (110). Due to their low affinity for the binding proteins, two-thirds of synthetic steroids binds weakly to albumin, while the remaining third circulates as free steroids (110, 111). Synthetic glucocorticoids have variable half-lives that are usually longer than that of endogenous cortisol (112,113). The half lives range from about one hour for prednisolone to over four hours for dexamethasone, (113-114).

Clearance is slower in older adults as compared to the younger population groups, which might explain the higher incidence of side effects in the elderly population (115).
Those subjects who metabolize glucocorticoids more slowly may be more likely to develop side effects (116), but whether there are distinct subgroups is uncertain. In addition to genetic variability, other drugs can influence the plasma clearance rate of steroids. CBG binding is not a major determinant of plasma half-lives for synthetic steroids.

Dexamethasone is one of the most successful and widely used anti-inflammatory agents. Its therapeutic indications apply to the adult and the pediatric populations alike. In children, it is commonly used in the treatment of airway edema, croup, cerebral edema, spinal cord compression, meningitis among others. In adults, it is used in the management of shock, chemotherapy-induced nausea and vomiting, and a wide range of inflammatory and allergic and immunological pathologies. Dexamethasone has the highest glucocorticoid activity of all synthetic corticosteroids.

Exogenous glucocorticoids undergo similar hepatic metabolism as endogenous steroids. Certain drugs such as phenytoin, rifampin and phenobarbital, activate the hepatic CYP3A4 enzyme and cause a decrease in the bioavailability of glucocorticoids (17=117) On the other hand, inhibition of CYP3A4, potentiates the effects of glucocorticoids, namely dexamethasone and methylprednisolone (18=118).

The relative anti-inflammatory and mineralocorticoid activities of synthetic glucocorticoids are two clinically important measures that govern the day-to-day use of glucocorticoids, depending on the outcomes desired by the clinician. When compared to

endogenous cortisol (relative anti-inflammatory activity of 1, and relative mineralocorticoid activity of 1), dexamethasone was found to have a relative anti-inflammatory activity of 30, the highest among all synthetic glucocorticoids, and a relative mineralocorticoid activity of zero, the lowest among its cousin molecules (119-120). These intrinsic properties of dexamethasone make it a very suitable compound to use when anti-inflammatory effects are desired, while minimizing the undesired mineralocorticoid effects.

The anti-inflammatory effects of glucocorticoids are achieved by several molecular mechanisms, which have been identified by basic research, which were discussed in an elegant milestone review article by Rhen and Cidlowski in 2005 (121). The authors described the three main mechanisms of glucocorticoid anti-inflammatory activity that have been previously identified in the literature. First, they described a direct effect on gene expression through binding of glucocorticoid receptors to specific glucocorticoid-sensitive elements. Secondly, they highlighted the indirect effects on gene expression by the interaction of glucocorticoid receptors with other factors, and underlined the glucocorticoid receptor–mediated effects on second-messengers.

2.4.2 Systemic side effects and toxicities

The therapeutic effects of glucocorticoids against inflammation are often accompanied by clinically significant side effects. This is unfortunately because some of these mechanisms are also involved in normal physiologic signaling pathways, in addition to the inflammatory signaling pathways. It has been shown that the cumulative dose and the length of glucocorticoid treatment are strongly associated with increased incidence of side effects, on almost all organ systems.

Adrenal gland atrophy, iatrogenic Cushing syndrome and weight gain represent the hallmark of long-term glucocorticoid therapy. These features result from the inhibition of the hypothalamic-pituitary axis and the redistribution of body fat. Weight gain can also be due to an increase in appetite and food intake for symptomatic relief in patients with gastropathy or peptic ulcer disease (122).

Hypertension has been shown to result from two distinct mechanisms: one involving renal sodium retention, giving rise to an expansion in blood volume; and a second implicating the induction of angiotensin II receptors, which leads to increased vasopressor responses to angiotensin II and catecholamines (123).

Furthermore, long term or high-dose glucocorticoids treatment is associated with an increased risk of osteoporosis and fractures (124). Osteoporosis is partly mediated by the glucocorticoid inhibition of osteocalcin transcription in osteoblasts. The absence of this important extracellular matrix protein that promotes bone mineralization, results in frail bones that are more prone to pathological fractures (125-126). In addition, glucocorticoids aggravate osteoporosis by inducing osteoblast apopotosis and potentiating osteoclastic activity. Osteonecrosis is an independent entity that manifests as ischemic or avascular necrosis of the bone, and occurs with high dose glucocorticoids

Additionally, glucocorticoids impair adequate wound healing by blocking cytokine signaling, thus inhibiting the synthesis of matrix metalloproteinases and collagen, which are major players in wound repair (127-130). Simultaneously, glucocorticoids induce hepatic gluconeogenesis through the degradation of proteins in muscle tissue, leading to muscular atrophy and ultimately causing hyperglycemia (131-133)

The skin is subject to one of the most common glucocorticoid toxicities: skin thinning and purpura. While in one study by Fardet et al, skin thinning was present in 46% of patients who took oral prednisone at doses greater than of 20 mg daily for three months (134), purpura was only seen in 32 patients out of 1000 in a large arthritis cohort (135). Increased systemic vascular resistance, with the ensuing hypertension, is one of the major side effects of longstanding glucocorticoid therapy. Other musculoskeletal side effects include proximal motor muscle weakness in upper and lower extremities. Myopathy is however less frequent than the previously discussed musculoskeletal events.

Acting on the central nervous system, glucocorticoids induce a range of psychiatric and cognitive symptoms, which are dose and duration-dependent (136). In the majority of patients, these symptoms are mild, reversible and self-remitting.

New-onset diabetes in patients with previously normal glucose tolerance is fairly uncommon (137). Diabetic patients exhibit higher glucose levels when started on glucocorticoids, and a closer monitoring and control is generally warranted in this population. Rarely hyperosmolar state or diabetic ketoacidosis may develop in patients with subclinical diabetes (138).

The mechanisms by which hyperglycemia develops include, alteration of receptor and post-receptor functions, activation of hepatic gluconeogenesis and inhibition of glucose uptake in adipose tissue. Although some patients may progress to develop overt type-2 diabetes, the condition improves with dose reduction and is usually reversible (139,140).

The immune system is also a target of glucocorticoids side effects. Systemic steroids affect the innate and acquired immune systems and predispose the patient to infection by bacterial, viral, and fungal pathogens. The mechanisms by which glucocorticoid therapy induces immunosuppression are complex. However, it important to consider the additional risk of immunosuppression that glucocorticoids may exert on patients receiving chemotherapy. Furthermore, those patients with lower functional status are also at higher risk for infection, as they may not manifest the associated signs and symptoms, due to their inability to mount an adequate inflammatory and febrile response.

In the pediatric population, the effects of glucocorticoids are mainly manifested by a delay in longitudinal growth. This is the result of inhibition of chondrocyte proliferation by alteration of the insulin-like growth factor signaling pathway, as well as apoptosis induction (141). Apoptosis in chondrocytes is mediated by the suppression of the Akt signaling pathway. Although longitudinal growth witnesses a period of catch- up growth once glucocorticoid therapy is discontinued, a continuous, high dose treatment of glucocorticoids during childhood is associated with overall decreased adult stature (141).

The material above has overviewed the most common and major side effects of systemic glucocorticoid therapy. The next section will overview the intra-tympanic route of glucocorticoid therapy administration, and will examine the associated benefits and side effects of intratympanic dexamethasone in the prevention of platinum-induced ototoxicity in animals. This

will complete our literature review, and will describe the last piece of the puzzle in our quest to better understand ototoxicity of platinum compounds and their interaction with dexamethasone therapy.

2.4.3 Dexamethasone for prevention of platinum ototoxicity in animal studies

The McGill Auditory Sciences Laboratory has had a rich and diverse experience in studying ototoxicity at the molecular level, using different ototoxic medications in more than one animal models, and assessing the protective effects of an extensive array of potential otoprotectant agents. In fact, researchers from this laboratory have improved understanding of the role of inflammation and oxidation in cisplatin-induced ototoxicity by using validated guinea pig and chinchilla animal models. They have also tested the otoprotective effect of 14 anti-oxidant and anti-inflammatory agents. Amongst all anti-inflammatory agents tested, they found dexamethasone to be the most effective at protecting against cisplatin ototoxicity in vivo (142). Murphy and Daniel's work constituted the first published study that used high-frequency Auditory Brainstem Response measurements to test the effects of dexamethasone against cisplatin ototoxicity in the guinea pig model. At the conclusion of their study, the authors also determined that the protective effect of dexamethasone on hearing thresholds was frequency and dose-related, and that dexamethasone presented a safe, simple and effective treatment modality to minimize cisplatin ototoxicity (142). After this milestone study, four other animal studies were conducted in three different animal models: guinea pigs, rats, and mice. These studies also examined the efficacy of intratympanic dexamethasone against cisplatin ototoxicity (142-146). All of these studies have shown a significant effect in preventing, or at least decreasing, ototoxicity.

2.5 Linking statement to the manuscript

Having reviewed the ear's anatomy, the pathophysiology of hearing loss, the platinum-based chemotherapeutic agents and their mechanisms of ototoxicity, as well as glucocorticoids and their potential role in otoprotection, Chapter 3 will examine the existing literature on intratympanic dexamethasone's efficacy in the treatment of sensorineural hearing loss in humans, as well as its side effect profile, through a systematic review and a meta analysis of the literature.

3- Manuscript 1- Efficacy and Safety of Intratympanic Dexamethasone in the Treatment of Sensorineural Hearing Loss in Adults: A Systematic Review and Meta-Analysis of Randomized Controlled Clinical Trials

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3.1 Abstract

Objective: Systemic dexamethasone has demonstrated conclusive benefits in reversing Sensorineural Hearing Loss (SNHL) despite considerable potential side effects. In contrast, the intratympanic route of steroid administration averts several possible complications. This study aims to examine the literature to delineate the efficacy and side effect of intratympanic dexamethasone injection (ITD) for the treatment of SNHL.

Data source: Cochrane, Embase and Medline electronic databases from January 1950 to August 2014 with an update performed on November 10, 2014.

Methods: Systematic review and meta-analysis of randomized controlled clinical trials, using the PRISMA flow diagram and guidelines. Quality assessment was performed using The Cochrane Collaboration's Tool for Assessing Risk of Bias.

Results: 12 RCCTs were included, 4 on Menière's Disease (MD) and 8 on Sudden Sensorinneural Hearing Loss (SSNHL). 50% of the studies had high risk of bias. Substantial heterogeneity was found. The meta-analysis failed to detect statistically significant difference between ITD and alternative treatment (OR=0.39, 95% CrI= 0.13-1.07). Subgroup analysis revealed similar odds ratios for MD and SSNHL. The side effects profile was favorable for ITD. No serious adverse events were recorded.

Conclusion: We found no evidence to support a difference between ITD and alternative therapy for SNHL. We recommend larger RCCTs to determine the effectiveness of ITD compared to oral steroid therapy. We encourage a shift in study design selection towards non-inferiority or superiority studies. Avoiding systemic corticotherapy, especially in vulnerable populations, should be the rationale for future research in the field.

Keywords: intratympanic, sensorineural hearing loss, middle ear, steroids, dexamethasone, injection, treatment, SSNHL, Menière.

3.2 Introduction

The etiologies of sensorineural hearing loss (SNHL) vary widely by patient population, underlying mechanism and propensity to be reversed by timely medical intervention. Among the pathologies widely studied during the last few decades are the idiopathic sudden-onset type of sensorineural hearing loss (SSNHL) and Menière's disease (MD). The interest in researching treatments for these diseases lies in the two major defining characteristics: relatively high prevalence (5-20/100,000 per year in SSNHL (147) and 4-46/100,000 in MD (148)) and tendency to be reversed. The majority of the treatment protocols developed for these pathologies are centered on glucocorticoids (149-161) due to their antioxidant and anti-inflammatory properties. Although systemic glucocorticoids demonstrated conclusive benefits in reversing SNHL (162-166) many clinicians are reluctant to administer these medications given their potential adverse effects. These include partial inhibition of the Hypothalamic-Pituitary-Adrenal axis in up to 40% of patients on oral prednisolone following a short course of prednisolone treatment (167), increasing the risk for adissonian crisis in the setting of physiological stress such as an acute illness or a surgery. Other potential adverse events include osteoporosis, hyperglycemia, hypertension and osteonecrosis at cumulative doses of 80-160mg of oral methylprednisolone (168).

The intratympanic (IT) route of steroid administration to the inner ear for the treatment of SSNHL and MD is a promising technique that allows for the delivery of small amounts of steroids to the inner ear while simultaneously bypassing the adverse events of the systemic route; it also allows for a higher steroid concentration in the perilymphatic fluid (167,169,170). In one large multicenter Randomized Controlled Clinical Trial (RCCT) that compared oral vs. IT steroids, equal hearing improvement was observed in the two groups (172).

Although many steroid preparations, concentrations and injection techniques have been explored (3-15), a consensus has not been reached on the indications for IT steroids. Well-defined concentrations, dosage and a standardized treatment protocol remain elusive. Thus, we sought to systematically review the literature on the efficacy and side effects of IT-dexamethasone (ITD) in the treatment of SNHL, the glucocorticoids of choice recommended by the American Academy of Otolaryngology Head and Neck Surgery (AAO-HNS) for the treatment of SNHL (173,174)

3.3 Methods

With the assistance of two medical librarians, articles were identified through a comprehensive search of the Cochrane, Embase and Medline electronic databases from January 1950 to August 2014. A search update was performed on November 10, 2014. The search strategy included medical subject headings (MeSH) and sub-headings; keywords included "intratympanic", "sensorineural hearing loss", "sudden sensorineural hearing loss", "Menière", "steroids", "dexamethasone", "injection" and "treatment".

We included only RCCTs of adults that compared the treatment of SNHL with ITD (treatment group) to another modality (control group) i.e.: oral steroids, intravenous steroids, hyperbaric oxygen or normal saline placebo. We included both first and second-line ITD studies that were published in English or French. The included studies had to report a well-defined efficacy parameter of hearing improvement (expressed in Pure Tone Average).

Studies were excluded if they did not state the name of the drug used, did not describe the method of ITD injection, or did not report the numbers of patients with successful outcome. Studies with simultaneous combined modalities of therapy were excluded. Editorial letters, conference proceedings, non-randomized observational studies, cohorts and retrospective studies were excluded.

Two authors (NES and AB) independently reviewed the titles and abstracts retrieved by the electronic search, and removed studies not concordant with the eligibility criteria. Reasons for exclusion were recorded and crossed-checked for agreement. Disagreements were resolved by consulting the senior author. The relevant articles underwent second stage review and were examined as full texts to revalidate inclusion. To complete our search, hand searching was performed on the references of the included articles to identify additional studies that may have been missed.

The following data were extracted: study country of origin, treatment and control group size, dosing regimen and total cumulative dose of ITD received, condition treated, mean age of participants, first line or second-line therapy, duration of follow up, definition of outcome measures, adverse events, and hearing outcome (reported as Pure Tone Average).

Definition of improvement: Due to differences in reporting hearing outcomes, we opted to invoke the recommendations for outcome assessments in the AAO-HNS clinical practice

guidelines (26). For the studies on MD, the Committee on Hearing and Equilibrium guidelines did not include any standardized hearing-related outcome measure (174). Therefore, to homogenize the reporting of hearing outcomes, we applied the same criteria.

According to the AAO-HNS complete recovery is defined as a return to within 10-dB HL of the unaffected ear AND recovery of word recognition scores to 5-10% of the unaffected ear. Partial recovery is defined as a return of the hearing in the affected ear that was rendered non-serviceable after the SSNHL event to a serviceable state (the ear is candidate for traditional hearing amplification). For an ear with SSNHL that is still in the serviceable range, a 10-dB HL improvement or an improvement in WRS of 10% or more should be considered partial recovery. Any improvement less than 10-dB HL is considered as no recovery (173).

Quality assessment: The methodological quality of the included RCCTs was assessed using the Cochrane Collaboration's tool for assessing risk of bias (175). Two reviewers (NES and MS) performed the quality assessment of studies.

Statistical analysis and synthesis: Differences in study methods, patient characteristics, and practice patterns suggest that the effects of the treatment are likely to vary from study to study. We therefore used a Bayesian hierarchical (random effects) model, which accounts for between-study variations in odds ratios. At the first level of this hierarchical model, the probability (p) of an outcome within each group of each study varies. In particular, the logarithm of the odds ratio for the outcome is assumed to have a normal distribution. The mean of the normal distribution of log-odds ratios across studies represents the average effect in the studies, and the variance of the normal distribution represents the between-study variability. Low-information prior distributions were used throughout, so that the study data drives the final inferences. WinBUGS software, version 1.4.3 (MRC Biostatistics Unit, Cambridge, UK) was used for analyses. Forest plots were produced to display the OR and 95% credible intervals (CrIs) for all major outcomes pooled in our meta-analysis. Credible intervals are the Bayesian analogue to frequentist confidence intervals.

Definition and classification of side effects: Given the absence of a classification scale for IT treatment side effects, our group decided to separate them into 4 different groups: The first group included procedure-related, very short-term, self-resolving side effects. The second group included procedure-related, short-term side effects, requiring medical or surgical interventions.

The third group included procedure-related, long-term side effects, requiring medical or surgical interventions. The fourth group included any drug related side effect.

3.4 Results

The literature searches yielded 933 articles, of which 508 (54.4%) were duplicate citations. The remaining 425 citations were screened for relevance and 314 (73.9%) of which were irrelevant and excluded, yielding 111 articles. The updated literature search added 9 new articles. These 120 articles were then assessed for eligibility, and 108 were subsequently excluded (Figure 3.1), yielding a total of 12 RCCTs for analysis.

FIGURE 1: Study PRISMA flowchart

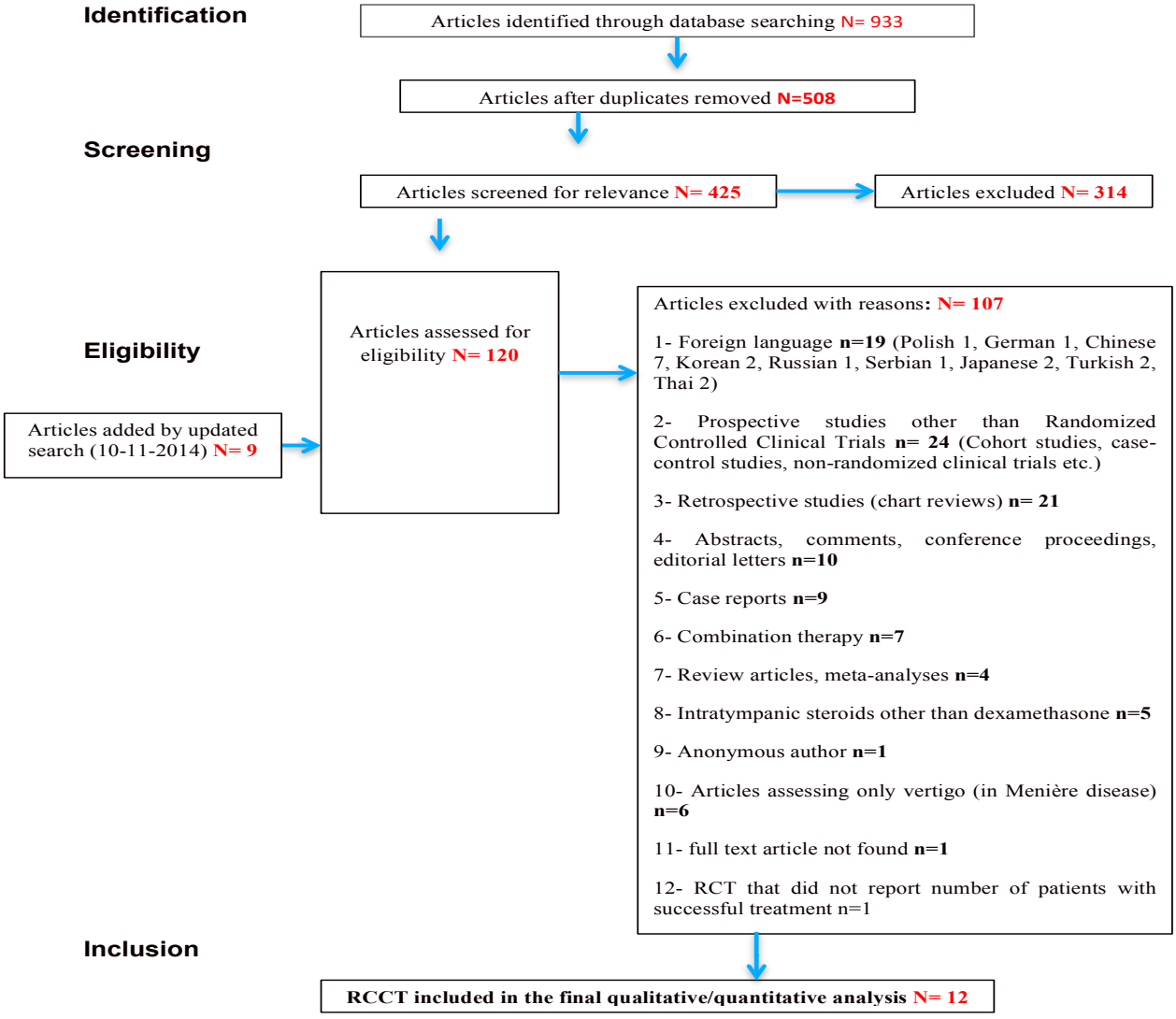

Figure 3.1 Search strategy for published randomized clinical trials on the treatment of sensorineural hearing loss with intratympanic dexamethasone injections. Data sources used were MEDLINE, EMBASE and COCHRANE (through November, 2014).

Condition: The underlying medical condition behind SNHL was MD in 4 studies and SSNHL in 8 studies.

Control groups: Of the 4 studies on MD, 2 used normal saline IT-injections as placebo (176,177), 1 used conventional therapy (178) and 1 used intravenous gentamycin (179). Of the studies on SSNHL, five used oral steroids and/or combination therapy in their control groups (176, 180-184), two used IT normal saline as placebo (185,186). In 1 study on SSNHL, the authors used their institution's standard treatment modality as control, consisting of a vasodilator, a benzodiazepine and vitamin B complex (187).

Treatment protocols: The concentrations of ITD ranged from 0.2 mg/ml (178) to 16 mg/ml (188). The mode concentration was 4 mg/ml and was used in 5 studies (176,179,185-187) followed by 5 mg/ml in 3 studies (180-183)

The dosing regimens of ITD also varied among the studies, and seemed arbitrary in the absence of justification for the choice of ITD dosage. In SSNHL studies, the most condensed dosing regimen consisted of one ITD injection/day for 8 consecutive days (179). Other regimens varied from weekly ITD injections for two weeks (188), three weeks (178,187) or four weeks (176). One study had a twice a week for two weeks (182) and another a three-times a week for two weeks regimen (183). In one study the mode of administration was a continuous infusion through a round window catheter applied for 14 days (186). In the MD studies, one study administered one ITD injection every three days for a total of three injections (179). In one study, patients were instructed to self-administer the ITD dose daily in the form of eardrops through ventilation tubes for a period of three months (178).

In contrast, the delivery technique to the middle ear cavity was more consistent and homogenous among the different RCCTs. In all studies, patients were put in the "otologic position", and applied topical anaesthesia to the external acoustic canal and the tympanic membrane (TM) (Emla cream or xycolcaine spray). In 9 studies a single myringotomy was performed using spinal needles between 22 and 27 gauge in size. Of the remaining 4 studies, one performed myringotomy and placed ventilation tubes (178), while another used an implanted round window catheter for continuous infusion (186), a third performed two myringotomies on the TMs (one for ventilation and the other for injection) (34) and the fourth performed laser assisted tympanostomy (184). The volume of ITD injected into the middle ear varied between

0.3 and 0.8 ml. In 10 studies the myringotomies were performed in the posterior inferior quadrant and in 2 studies, in the anterior superior quadrant (180,183).

Eleven studies discussed their follow-up on patients after the completion of treatment. The follow-up period varied from 2 weeks (186) to 2 years (179).

The total number of participants across the studies was 551 with 259 patients (47.0%) in the treatment groups and 292 (53.0%) in the control groups. There were 210 men (44.5%) and 262 women (55.5%) who participated in 9 studies but 3 studies did not report the participant gender ratio (178,184,186). The mean age of participants ranged between 38.5 and 61.5 years. The characteristics of studies and participants are shown in table 3.1

Author	Country	Intervention	Treatment and Control groups	Average age	Condition	ITD concentration	Follow up	Side Effects (n)	Definition of Outcome Measures	Hearing Outcome	Conclusion
Paragache (2005)	India	Self ear drops through Ventilation tubes (×3 months)	ITD:20 Conventional theapy:20	38.5	MD (1st line)	0.2mg/ml	6mo	N/M	Increase in hearing thresholds (>10dB)	>20dB: 2 (10%) >10 dB: 1 (5%) Total: 3 (15%)	Patients with shorter duration of disease had increased benefit by ITD therapy.
Casani (2012)	Italy	3 injections (1 every 3days)	ITD: 26 ITG: 31	53.7	MD (salvage)	4 mg/ml	2 y	N/M	AAO HNS 1995	No deterioration: 13 (46.42%) Improvement: 3 (10.7%) Worsening: 10 (38.4%) Mean hearing improvement: 0.5dB	Not statistically significant for improvement of hearing loss
Dispenza (2011)	Italy	4 injections	ITD: 25 Oral Steroids:	47	SSNHL (1st line)	4 mg/ml	6 mo	N/M	>10dbB improvement	Improvement: 20 (80%)	Equal results between systemic therapy and

											first line treatment
Ho (2004)	Taiwan	3 injections (1/week)	ITD: 15 Standard therapy: 14	N/M	SSNHL (salvage)	4 mg/ml	2.6 mo	Vertigo (1) Acne (1)	No recovery: PTA improvement <10dB Slight: PTA improvement >10dB and <30dB Moderate: PTA Improvement>30dB Complete: PTA<25dB	Complete: 4 (26.6%) Moderate 4 (26.6%) Slight: 3 (20%) Total: 11 (73.3%) No recovery: 4 (26.6%) Mean hearing improvement: 28.4dB	ITD effectively improves hearing in patients with severe or profound SSNHL
Hong (2009)	Korea	8 injections (1/day)	ITD: 32 Oral steroids: 31	56.9	SSHNL (1st line)	5 mg/ml	N/M	None (0%)	Siegel criteria (No, slight, partial, complete)	Complete:10 (32%) Moderate: 7 (22%) Slight: 8 (25%) No recovery: 7 (22%) Mean hearing improvement: 27dB	Daily ITD as primary treatment modality is effective for the management of idiopathic SSNHL (as effective as OS)
Lim (2013)	Korea	4 injections (2/week)	ITD: 20 ITD+ Oral steroids: 20 Oral steroids: 20	53.3	SSNHL (1st line)	5 mg/ml	3 w	N/M	AAO-HNS 2012	Recovery: 11 (55%) Complete 3 (15%) Partial 8 (40%)	Systemic and/or local steroid therapy can be suggested as an initial treatment in idiopathic SSNHL.
Park (2011)	Korea	6 injections (over 2 weeks)	ITD: 44 Simultaneous IVS/oral steroids + ITD: 44	48.05	SSNHL (1st line)	5mg/ml	3 mo	Otalgia (11), Transient dizziness (9), ear fullness (8), headache	Siegel Criteria	Complete: 17 (38.6%) Partial: 8 (18.2%) Slight: 9 (20.5%) No recovery: 10 (22.7%) Mean hearing improvement: 32.59 dB	No difference between simultaneous and subsequent ITD therapy

								(5), TM perforation (2), otorrhea (4)			
Plontke (2009)	Germany	Round window catheter Total dose: 0.58 mg	ITD: 12 ITNS: 11	56	SSNHL (salvage)	4 mg/ml	2 w	Otalgia (2) Headache (1) Ear canal skin defect (1) Vertigo (1)	Ho Classification and changes in PTA and Speech Discrimination Score	ITD: 6/12 (50%) improved >10dB Mean hearing improvement: 13.9 dB	No statistically significant hearing improvement. Encourages further investigation of this treatment
Wu (2011)	Taiwan	4 injections (over 2 weeks)	ITD: 27 ITNS: 28	49.1	SSNHL (salvage)	4 mg/ml	1 m	Dizziness (18), TM perforation (1)	Any PTA improvement	>20dbB: 4 (14.8%) >15dB: 8 (29.6%) >10dB: 12 (44.4%) Mean hearing improvement: 9.8dB	ITD is audiologically beneficial for SSHL after the failure of initial systemic steroid therapy.
Silverstein (1998)	USA	3 injections	ITD: 10 ITNS: 7	N/M	MD (1st line)	16 mg/ml	7 w	N/M	AAO HNS 1995	ITD: 2/10 (20%) improved >10dB ITNS:0/7 (0%) improved >10dB Mean hearing improvement +0.6dB (Worsening)	ITD does not differ from placebo

Battaglia (2008)	USA	3 injections (1/week)	ITD: 17 Oral steroids: 18 Conventional therapy: 16	60	SSNHL (1st line)	12 mg/ml	3 m	None (0%)	Complete: PTA within 5 dB of the contralatera l ear Partial: decrease of 15 dB	Partial: 5(29%) Complete: 5 (29%) Mean Hearing Improvement: 30dB	CT>ITD alone
Garduño-Anaya (2005)	Mexic o	4 injections (1/day)	ITD: 11 ITNS: 11	50	MD (Salvage)	4 mg/ml	2 y	N/M	Secondary outcome: AAO HNS 1995 (Menière guidelines)	>10db: 1 (9%) Deterioration: 1 (9%) 2-year follow-up average PTA= 53.4 dB	Good for vertigo but not for hearing loss

Table 3.1 Demographic data of the randomized clinical trials included in the meta-analysis

Quality Assessment

We used the Cochrane Collaboration's tool for assessing risk of bias (175), and added one criterion that we judged important for the assessment bias risk: the Intention To Treat Analysis (ITT). ITT maintains the integrity of the RCT. The results are presented in Table 3.2

Study	How was allocation sequence generated?	How was allocation sequence concealed?	What measures were taken to blind participants and personnel?	What measures were taken to blind outcome assessors?	Is the outcome data complete? Did the authors report exclusion and attritions and give reasons for these?	Is there the possibility of selective outcome reporting?	Are there any other potential sources of bias?	Summary assessment of risk of bias?	Was the Intention-To-Treat Analysis conducted?
Paragache (2005)	UNCLEAR The randomization was not mentioned at all	UNCLEAR Not described	HIGH It is clear from the description that the patients knew whether they were taking ITD or conventional therapy	UNCLEAR Not described	LOW The outcome data is complete. There were no exclusions or attritions	UNCLEAR The authors did not clearly specify the desired outcome measures. Hearing outcomes not defined and not adequately reported	HIGH It seems to us that this study is a retrospective observational study camouflaged into a prospective design. There is also major concern regarding the compliance of patients to self-administered ear drops for 3 months	HIGH	YES
Casani (2012)	LOW Authors clearly mention the use of a computer software to generate the randomization sequence	UNCLEAR Not described	UNCLEAR Study did not address	UNCLEAR Not described	LOW The outcome data is complete. Authors clearly describe 3 patients lost to follow-up at 2 years, and 1 patient considered as failed treatment at 1 year, who underwent definitive surgical treatment	LOW All endpoints are reported	LOW None	LOW	NO

Silverstein (1998)	UNCLEAR Authors mentioned randomization but did not specify the methods used	UNCLEAR Not described	LOW Authors mentioned blinding but did not describe measures	LOW Authors mentioned blinding but did not describe measures	LOW The outcome data is complete. Authors clearly describe 2 patients lost to follow-up before the second arm of the study	LOW All endpoints are reported	LOW None	LOW	NO
Garduño-Anaya (2005)	UNCLEAR Authors mentioned randomization but did not specify the methods used	UNCLEAR Not described	LOW Authors mentioned blinding but did not describe measures	LOW Authors mentioned blinding but did not describe measures	HIGH Authors describe 4 patients from the control group not analyzed at the end of the 2-year follow up: 3 classified as failed treatment and offered another treatment, and 1 patient abandoned the study	LOW All endpoints are reported	LOW None	HIGH	NO
Dispenza (2011)	UNCLEAR Authors mentioned randomization but did not specify the methods used	UNCLEAR Not described	UNCLEAR Study did not address	UNCLEAR Not described	LOW Authors describe 3 patients lost to follow up and 2 diagnosed with vestibular Schwannoma by MRI	LOW All endpoints are reported	LOW None	LOW	YES

Ho (2004)	UNCLEAR Authors mentioned randomization but did not specify the methods used	UNCLEAR Not described	HIGH It is understood from the methods section that participants were not blinded	HIGH It is understood from the methods section that assessors were not blinded	LOW The outcome data is complete. There were no exclusions and no attritions	LOW All endpoints are reported	LOW None	HIGH	YES
Hong (2009)	UNCLEAR Authors mentioned randomization but did not specify the methods used	LOW Alternate sequence allocation mentioned	HIGH It is clear from the description that the patients knew whether they were taking ITD or conventional therapy	LOW Authors explicitly mentioned blinding of assessors but did not provide description of how the blinding was achieved	LOW Authors identify in a flow diagram the patients that were excluded from the study or lost to follow-up. The numbers are evenly distributed between the two arms of the study	LOW All endpoints are reported	LOW None	HIGH	NO
Lim (2013)	LOW Authors clearly mention sequence generation procedure	UNCLEAR Not described	HIGH It is understood from the methods section that participants were not blinded	HIGH It is understood from the methods section that assessors were not blinded	LOW Authors identify in a flow diagram the patients that were excluded from the study or lost to follow-up. The numbers are evenly distributed between the two arms of the study	LOW All endpoints are reported	LOW None	HIGH	YES

Park (2011)	LOW Authors clearly mention sequence generation procedure (SPSS)	UNCLEAR Not described	UNCLEAR Study did not address	LOW Authors explicitly metioned blinding of assessors (audiologists)	LOW Authors identify in a flow diagram the patients that were excluded from the study or lost to follow-up. The numbers are evenly distributed between the two arms of the study	LOW All endpoints are reported	LOW None	LOW	NO
Plontke (2009)	LOW Authors clearly mention sequence generation procedure	UNCLEAR Not described	LOW Study design implies that participants were adequately blinded since both arms received the same intervention	UNCLEAR Not described	LOW Authors identify in a flow diagram the patients that were excluded from the study or lost to follow-up. The numbers are evenly distributed between the two arms of the study	LOW All endpoints are reported	HIGH The nomenclature of "simultaneous and subsequent" implies all patients received standard therapy within the window of ITD treatment, which limits the homogeneity between the study arms	LOW	YES

Wu (2011)	UNCLEAR Authors mentioned randomization but did not specify the methods used	UNCLEAR Not described	UNCLEAR Study did not address	UNCLEAR Not described	LOW Authors identify in a flow diagram the patients that were excluded from the study or lost to follow-up. The numbers are evenly distributed between the two arms of the study	LOW All endpoints are reported	LOW None	LOW	NO
Battaglia (2008)	UNCLEAR Authors mentioned randomization but did not specify the methods used	UNCLEAR Not described	LOW Study design implies that participants were adequately blinded since both arms received the same intervention	UNCLEAR Not described	LOW Authors clearly identify the patients excluded or lostto follow-up. The numbers are evenly distributed between the 3 arms of the study	LOW All endpoints are reported	LOW None	LOW	YES

Table 3.2 Assessment of risk of bias for RCCTs that compared ITD to any other treatment for SNHL. (Adapted from the Cochrane Collaboration's tool for assessment of risk of bias)

Efficacy

The efficacy (improvement in PTA) in MD-related hearing loss across all studies was 6% (4/67). All 4 patients who improved had only partial recovery. Casani et al. reported an average PTA improvement of 0.5 dB, which corresponded to no recovery, as previously defined. The efficacy rates are presented in Table 3.3.

Study	Improvement ITD arm	Improvement control arm	Treatment
Paragache (0.2mg/ml)	15%	10%	2nd line
Casani (4mg/ml)	0%	0%	2nd line
Silverstein (16mg/ml)	20%	0%	1st line
Garduño-Anaya (4mg/ml)	9%	0%	2nd line

Table 3.3 Efficacy of studies on ITD for Menière disease-related hearing loss.
*Statistically significant at $p < 0.05$

Wu et al. reported the highest efficacy rate (89%) for ITD as a second-line treatment for refractory SSNHL after first line treatment failure (185). The lowest efficacy rate observed was 50% in the study by Plontke et al. (186). Overall, hearing improvement was seen in 72% of all the patients randomized into the ITD treatment arms in the 8 studies. Complete hearing recovery was achieved in 20% of patients in the ITD treatment groups, 52% achieved partial hearing improvement and 28% showed no improvement. The efficacy rates by study are described in Table 3.4.

Study	Improvement ITD arm	Improvement control arm	Treatment
Dispenza (4mg/ml)	80%	8%	1st line
Ho* (4mg/ml)	73%	7%	2nd line
Hong (5mg/ml)	79%	75%	1st line
Lim (5mg/ml)	55%	60%	1st line
Park (5mg/ml)	77%	84%	1st line
Plontke (4mg/ml)	50%	27%	2nd line
Wu* (4mg/ml)	89%	11%	2nd line
Battaglia (12mg/ml)	58%	61%	1st line

Table 3.4 Efficacy of studies on ITD for SSNHL * Statistically significant at $p < 0.05$

Meta-analyses: We performed a Meta-Analysis on all 12 studies, and two subgroup analyses based on the underlying pathology and the performance of ITT analyses. Neither the overall Meta-analysis nor the subgroup analyses on the MD and SSNHL studies reached statistical significance. The Forest Plot of the overall meta-analysis is shown in figure 3.2. A large heterogeneity was noted among these studies. The results are described in the Forest plot (Figures 3.3, 3.4 respectively). There were also no statistically significant differences between studies that included ITT and those that did not. The ITT-negative group had an OR=0.27, 95%CrI (0.05-1.43), whereas the ITT-positive had an OR= 1.11, 95% CrI (0.14-1.60).

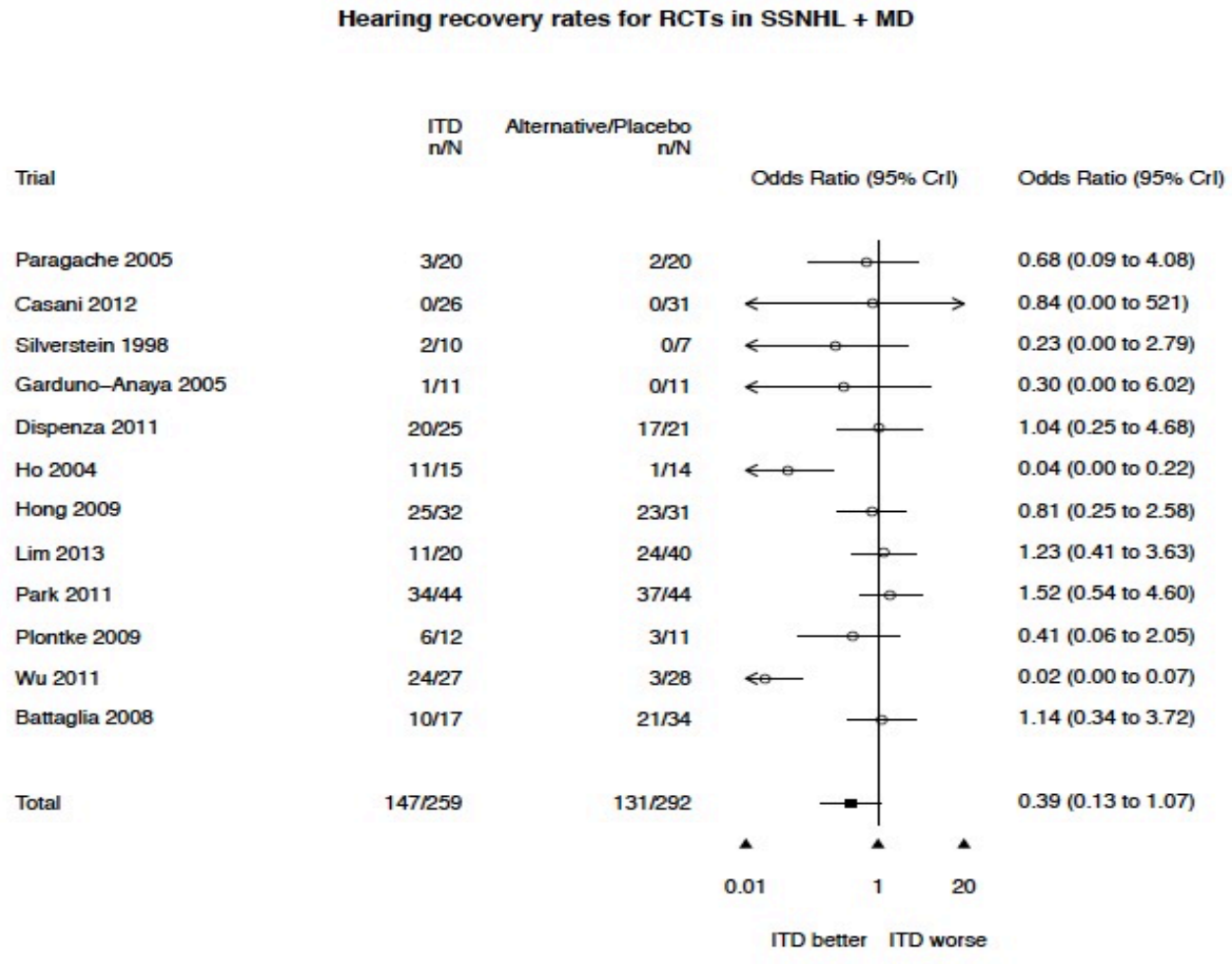

Figure 3.2 Hearing recovery rates for RCCTs in SSNHL and MD. (CrI: Credibility Interval, n: number of successes, N: sample size)

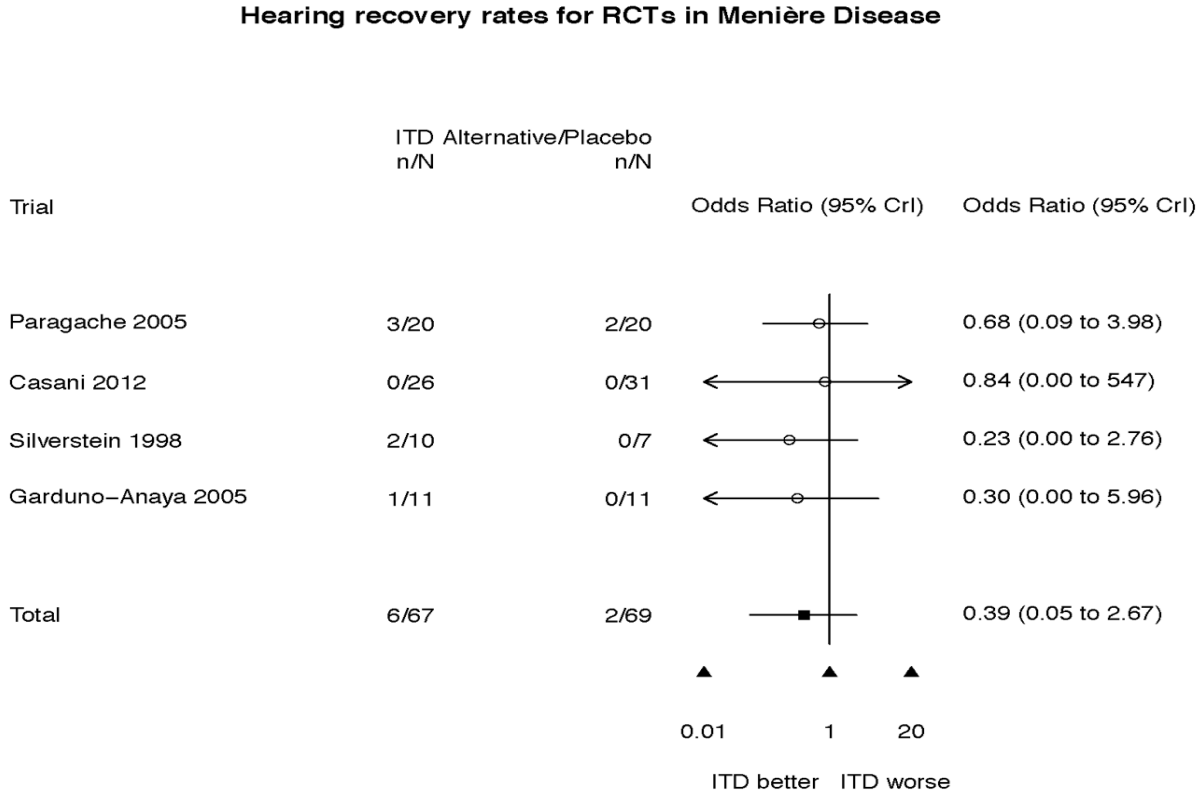

Figure 3.3 Hearing recovery rates for RCCTs in MD. (CrI: Credibility Interval, n: number of successes, N: sample size)

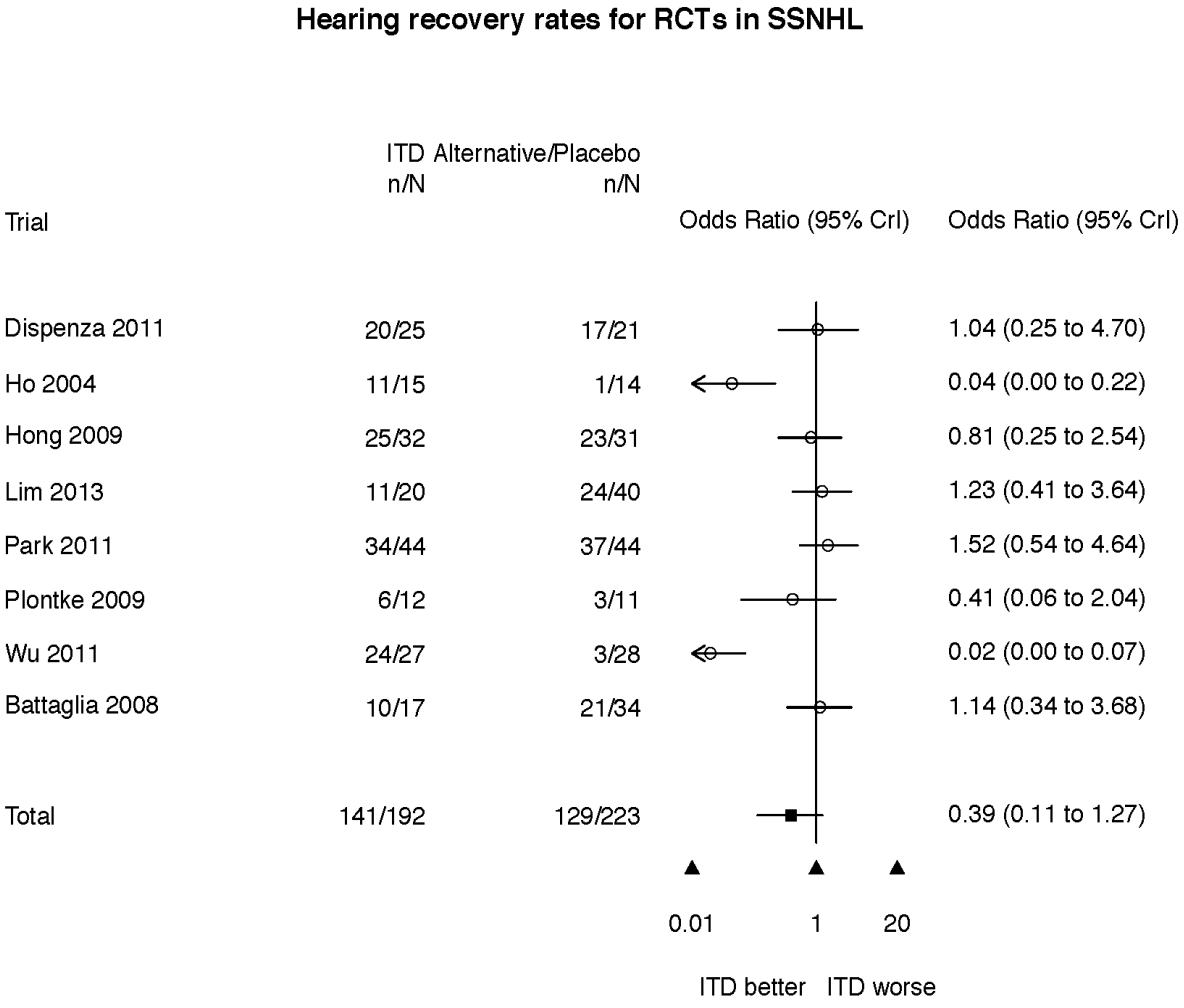

Figure 3.4. Hearing recovery rates for RCCTs in SSNHL (CrI: Credibility Interval, n: number of successes, N: sample size.)

Side Effects

Of the 12 included studies, 7 reported side effects: 0 on MD and 7 on SSNHL. We separated the side effects into the four groups based on the clinical criteria of time of onset and severity as described in the methods section. The first group included 57 side effects events, representing 81% of all side effects across 7 studies and affecting 16.8 % of the study population. The second group counted 5 side effects events: 3 severe dizziness events (185) and 2 perforations of the TM after injection, which resolved spontaneously at one-month follow up

(183,185). These represented 7% of all reported side effects and affected 1.5% of the patients across the 7 studies. The third group included 3 events: 1 case of otorrhea requiring topical and oral antibiotic treatment (183), and 2 cases of TM perforation requiring surgical repair by myringoplasty (183,186). These represented 4% of all side effects and affected less than 1% of the total population in the 7 studies. The fourth group (dexamethasone-related side effects) included one case of acne reported by Ho (187), and sporadic single cases of acne, gastroenteritis, hypokalemia and increased liver function tests reported by Plontke, who concluded that they were neither related to the intervention nor to dexamethasone (186). In total, 70 out of 339 patients (17.5%) experienced adverse effects, more than 87% of which was mild and self-resolving. There were no serious or life threatening side effects reported.

3.5 Discussion

Findings from our meta-analysis were inconclusive in addressing whether ITD is more effective at improving hearing loss compared to either standard therapy or placebo in the treatment of SNHL. There were a limited number of relevant studies, and the sample sizes within each study were small. We determined the risk of bias to be very high in 6 of the 12 studies mainly due to missing Intention-to-Treat analysis. In itself, the absence of this single parameter in more than 50% of the studies added to the overall high bias low quality of the included studies.

ITD for SSNHL: Although the meta-analysis showed no statistically significant difference between the ITD and the control groups, the absence of evidence is not evidence of absence. The wide confidence intervals and the weak and quantitative statistical analysis are due, in part, to the heterogeneity of these studies. This heterogeneity manifested at every level of the study design. However, the dosing regimens, injection techniques, dosages of ITD and follow-up windows rarely complied with the AAO guidelines. The variability in the nature of the control groups also contributed to heterogeneity. As shown in table 4, the control groups of the 12 RCCTs did not receive the same intervention. We homogenized the outcomes among studies by translating the reported outcomes into the AAO-HNS 2012 guidelines. The nature of the intervention (1st line v/s 2nd line) was another factor intrinsic to the study design that likely exacerbated the heterogeneity effect. A subgroup analysis on 1st v/s 2nd line treatments was not

feasible due to the very small number of studies.

Nevertheless, some interesting efficacy results were noticed. The Ho and Wu studies were the only two to show statistically significant results (185,187). Ho and colleagues compared ITD to conventional therapy as second line treatment, after failure of conventional therapy (oral steroids, vasodilators, vitamin-B complex, and benzodiazepine (187). Their results showed 73% improvement in the ITD arm compared to 7% in the control arm. Wu and colleagues' study compared ITD to IT normal saline, as a second line treatment after failure of primary oral steroid therapy (185). Their results described 89% improvement in the ITD arm compared to 11% in the control arm. Interestingly, these two studies shared some similarities such as 1) the use of ITD as a second-line treatment, 2) concentration of 4 mg/ml, and 3) close adherence to the regimen proposed by the AAO-HNS guidelines.

ITD for Menière disease: The fact that the pathophysiology of MD comprises two distinct symptoms (tinnitus and hearing loss), made it difficult for trials to clearly define outcome measures and assess efficacy of the intratympanic treatment. The four studies on MD included in this meta-analysis failed to show a difference between ITD and other conventional therapies. Interestingly, otologists tend to agree that, for MD, IT steroids seem to alleviate tinnitus but have a negligible effect on the associated hearing loss.

Given the limited available data on IT dexamethasone, we strongly encourage the implementation of large-scale randomized controlled trials from tertiary otology referral centers that have the ability to gather larger numbers of patients into one study. This centralization can eliminate most of the bias risks encountered, and guarantee no heterogeneity within the same study. Stratification of treatment groups could help compare different IT steroids to each other and to conventional therapy, or different groups of patients based on the severity of their disease. If IT dexamethasone or another steroid could be proven beneficial for MD-related hearing loss, invasive surgical interventions could be reserved to treat only the severe cases.

Quality Assessment: We used the Cochrane Collaboration's tool for assessing risk of bias (175) as the measure of quality, and added one criterion: the Intention-To-Treat (ITT) analysis. As a result, 5 studies were "high" on risk of bias (178,180,181,187,188). The most common source of bias was the performance bias: the absence of blinding of participants, personnel and assessors. Blinding of participants and personnel was absent in 4 studies (178,180,181,187). Nine studies failed to blind the assessors. Two studies had incomplete outcome data and failed to report exclusions, attritions, or give reasons for these. In one study, 4 patients from the control group were excluded for unknown reasons from the study after the 2-year follow up, violating the ITT rule, with vague justification of the reasons for the attrition. To allow for a larger sample size that compensate for large attrition rates, collaboration among referral centers is recommended as is following the PRISMA reporting guidelines.

Side effects: This systematic review is the first to assess side effects of IT steroids in adults. Adverse events often dictate the treatment modality and limit the implementation of new clinical trials in vulnerable populations. We believe that an IT steroids side effects scale, like the one we proposed in this study, can be helpful in quantifying IT steroid-related side effects and divide them according to time of onset, severity and underlying mechanism (procedure or drug-related), to help steer further research and unfold the full potential of IT steroids especially in the pediatric and other vulnerable populations. According to a prospective study from Switzerland, IT corticosteroids did not interfere with either endogenous cortisol secretion or bone metabolism, two highly glucocorticoid-sensitive endogenous systems that can detect minor interferences from exogenous steroid sources (187). Therefore the incidence of systemic side effects was expected to be negligible, which is concordant with the results we derived out of our side effect assessment.

The side effects were not systematically examined in all studies. None of the studies reported local outer, middle or inner ear side effects, whether related to external acoustic canal skin changes, middle ear ossicular disruption or thinning, or inner ear toxicity Dexamethasone-related side effects were virtually absent. The remaining three categories of side effects are technique-related. Despite them affecting 17.5% of the study population in the 12 studies combined, the majority of these events (81%) were technique-related, very short-term and self-

resolving. and included ear fullness, slight otalgia during injection, transient dizziness/vertigo post-injection, all of which can be attributed to the immediate injection technique and the pre-injection local anesthesia. We believe the transient vertigo attacks, given their resolution in just a few minutes, are the manifestation of the physiological vestibular "caloric test" that is due to introduction of warm or cold liquids into the external ear canal. A minority of side effects recorded required closer medical or surgical attention (second and third group). Two cases of persistent TM perforations were reported, to which patch repair was warranted one month after the procedure. Another case of TM perforation resolved spontaneously at the next follow-up visit. The dismal numbers of serious, locally aggressive adverse events indicates that the injection techniques used are mostly appropriate, and that the post-treatment follow-up and care are adequate in detecting these adverse events and promptly addressing them.

In their study, Plontke and colleagues (186) dismissed the relationship of these adverse events to the ITD therapy and the very unlikely systemic absorption of the drug. Thus we can affirm that, according to the retrospective and prospective studies examined, ITD is not systemically absorbed and does not lead to systemic and severe cortisol-related adverse events. It is therefore suitable to suggest that ITD therapy is a safe and reasonable procedure, and that dexamethasone injected intratympanically is not absorbed systemically and does not carry risks of cortisol-related metabolic or endocrine side effects. Furthermore, the slightly different techniques of ITD delivery described in the AAO-HNS 2012 guidelines are efficient in dispensing dexamethasone into the middle ear cavity. They remain however, surgeon-dependant.

3.6 Implications for clinical practice

As expected the quality of reporting of the clinical trials was not of the highest quality, possibly because they did not involve clinical epidemiologists. Most importantly ITT, which is an integral concept of therapeutic RCCT, in which all randomized patients' data should be analyzed at the end of the study, was only conducted in 6 of the 12 studies. We believe that, in order to produce RCCTs of higher quality, academic clinicians should pursue continuous medical education especially in the field of epidemiology and implement the highest standards of research methodology in the design, data collection and results interpretation.

This systematic review is the first to examine the efficacy and safety of ITD for SNHL. The inconclusiveness of the meta-analysis is due, in part, to the small number of RCCTs conducted to date and to the heterogeneity among studies. To address these issues in the future, we suggest collaboration among otolaryngology groups to implement a large multi-center clinical trial to compare routes of administration of dexamethasone (ITD and oral) as the treatment of choice, and their respective side effects profiles. Similar to what we did in this study, it could be helpful to the field to develop a side effect classification or scale of intratympanic injections based on "procedure vs. drug related", time of onset and severity. Furthermore, this is an excellent opportunity to design a study that reports on the side effect profiles in comparison to other treatment modalities.

It is advised that future researchers develop and assess various preparations of ITD, especially those that remain longer in the middle ear cavity and allow for a longer exposure time of the inner ear to dexamethasone, as research is beginning to show that exposure time has a much greater impact than concentration in achieving higher inner ear dexamethasone permeability (42). Finally, it is important to establish that IT-dexamethasone is non-inferior to systemic steroids as first line treatment of SNHL. If such non-inferiority is established, this will permit a shift in treatment approaches towards the less harmful IT route, especially if the side effect profile is favorable.

4- Discussion

4.1 Summary and discussion of the study findings and clinical implications

The treatment of soft tissue cancers in the pediatric population has witnessed an exponential development over the last two decades, and achieved significantly higher survival rates among children. This success is owed to the discovery and refinement of cisplatin, a chemotherapeutic agent that revolutionized treatment and survival of cancer simultaneously.

Unfortunately the price a child might pay for the cure of their cancer, in quality of life currency, can be substantial. Among all other cisplatin adverse effects, sensorineural hearing loss represents a peculiar and disastrous undesirable effect that plagues around 60% of children receiving this drug. More importantly, hearing loss is irreversible, dose-dependent and bilateral, which translates into severe socio-developmental retardation of the cancer survivors, especially at younger ages, when hearing is crucial for language and social skill acquisition. The result can be a lifelong hearing- and language-impaired child, at risk of social withdrawal, stigmatization, and poor school performance, who will carry the burden of this sequel and its epsilon consequences for the rest of his or her life.

From a socio-economic lens, the costs attributed to this crippling side effect are astronomical, and in light of the most recent healthcare budgetary austerity measures, investing in research aimed at discovering prevention, rises to the level of imminent necessity.

Many drugs have been sought to prevent cisplatin-induced ototoxicity. One drug in particular, dexamethasone, was found to have a protective effect in various animal models. Unfortunately no human studies have ever been conducted on an intra-tympanic formulation of dexamethasone, and therefore there is a lack efficacy and safety data in humans. Ethics boards in academic institutions are reluctant to give the green line for such studies in children before a clear side effect profile for an intra-tympanic formulation of dexamethasone is established.

This led to the work presented in this thesis to review the existing literature on the efficacy and safety of intra-tympanic dexamethasone, used in adults with sensorineural hearing loss and Menière’s disease. The scarcity of randomized clinical trials on this topic made the

analysis more difficult, and the derived results less and less powerful. More so, the studies that were examined, were of poor epidemiologic quality and extremely heterogeneous in their study populations, their interventions, their control groups, their follow up times, their dexamethasone concentrations used and their assessment tools. In addition, only a minority addressed the crucial issue of intratympanic-dexamethasone side effects, and did so in an unstructured and brief way.

Nevertheless, side effects reported were negligible in numbers and nature. The absence of major, glucocorticoid-related systemic side effects across these randomized clinical trials is promising. Dexamethasone solutions seem to remain in the middle ear cavity, and do not appear to diffuse into the systemic circulation. For the pediatric target population (children less than 18 years of age), this is a promising result. Despite these findings, care is required in interpretation of results, so that there are no rushed conclusions about the absolute safety.

4.2 Rationale for the clinical trial in children

In order to establish the safety of intratympanic dexamethasone in children, an adult study should be conducted first, with systemic side effects as the primary outcome measured. As an alternative, a basic science study at the molecular level should be designed, with the aim of assessing dexamethasone concentrations in the peripheral circulation after intra-tympanic injections. If either study obtains favorable results, intra-tympanic dexamethasone can then be considered as a medication for the pediatric population.

We are still taking our first steps in the journey of treatment of cisplatin ototoxicity in pediatric cancer patients, and our understanding of the molecular mechanisms of dexamethasone is yet to reach full maturity. However, the unquenchable thirst to discover a treatment for cisplatin-induced ototoxicity remains the driving force for generations of researchers in oncology and otolaryngology. Our team at the McGill Auditory Sciences Laboratory has laid down the foundations of a much-needed multicenter Canadian pilot study for the treatment and prevention of platinum-induced ototoxicity in pediatric cancer patients using intratympanic dexamethasone. The backbone of this CIHR funded proposal is formed by top quality research done by our lab, on the molecular mechanisms of cisplatin ototoxicity that have been cited worldwide. The

remaining question remains an ethical one, and indeed very eluding: if the benefits of such major study clearly outweigh the harms, the *Primum Non Nocere* oath is safeguarded in its spirit, and Ethics Review Boards ought not delay its implementation.

5- Conclusion

5.1 Claim of originality

This study is the first in the medical literature to examine randomized clinical trials on the efficacy and safety of intratympanic dexamethasone for the treatment of sensorineural hearing loss in humans. It contributes to the medical literature by shedding light on the field of intratympanic-dexamethasone injections in the adult population. Ultimately, these findings might motivate the conduct of much-needed clinical trials on the prevention of platinum-induced hearing loss in the pediatric population.

6- List of Abbreviations

AAO-HNS	American Academy of Otolaryngology, Head and Neck Surgery
ABR	Auditory Brainstem Response
AC	Air Conduction
BC	Bone Conduction
CBG	Corticosteroid Binding Globulin
CDDP	Cis-diamminedichloroplatinum (II)
CHL	Conductive Hearing Loss
CrI	Credibility Interval
CT	Conventional Therapy
dB	Decibel
DEX	Dexamethasone
DNA	Deoxyribo-Nucleic Acid
DPOAE	Distortion Product Oto-Acoustic Emissions
f	Sound Frequency
GBS	Group B Streptococcus
HL	Hearing Loss
Hz	Hertz
IHC	Inner Hair Cells
IT	Intra-Tympanic
ITD	Intra-Tympanic Dexamethasone
ITG	Intra-Tympanic Glucocorticoid
ITNS	Intra-Tympanic Normal Saline
IVS	Intra-Venous Steroids
KHz	Kilo Hertz
L	Sound level
MD	Menière's Disease
MeSH	Medical Subject Heading
N/M	Not Mentioned
OAE	Oto-Acoustic Emissions

OHC	Outer Hair Cells
OR	Odds Ratio
OS	Oral Steroids
PTA	Pure Tone Audiometry
RCCT	Randomized Controlled Clinical Trial
ROS	Reactive Oxygen Species
siRNA	Small Interfering Ribo-Nucleic Acid
SNHL	Sensori-Neural Hearing Loss
SSNHL	Sudden Sensori-Neural Hearing Loss
TM	Tympanic Membrane
TNF-alpha	Tumor Necrosis Factor alpha
WRS	Word Recognition Score

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