

**The role of extended preoperative steroids in hearing preservation
cochlear implantation**

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ABSTRACT

Introduction

Preoperative steroids have been shown to be beneficial in reducing the loss of residual hearing associated with cochlear implantation (CI). Previous studies have examined only the administration of steroids just prior to surgery. There are also currently no randomised controlled trials on the role of preoperative steroids in hearing preservation CI.

Aims

This thesis has three aims. Firstly, to review the mechanism of action, the effects of differing routes of administration, and the side effects of steroids administered to the inner ear. Studies on the role of preoperative steroids in animal and human studies will also be reviewed and future directions for research in this area will be discussed. The second aim is to examine the role of extended preoperative systemic steroids in hearing preservation CI in an animal model. The third aim is to determine if preoperative steroids, either via a transtympanic or systemic route, can improve hearing outcomes in CI in a human randomised controlled trial.

Methods

An animal model of CI was used. Hartley strain guinea pigs (n=24) with a mean weight of 768g and normal hearing were randomised into a control group, a second group receiving a single dose of systemic dexamethasone 1 day prior to surgery, and a third group receiving a daily dose of systemic dexamethasone for 5 days prior to surgery. A

specially designed CI electrode by Med-EL [Innsbruck] was inserted through a dorsolateral approach to an insertion depth of 5mm and left in situ. Auditory brain stem responses (ABR) at 8kHz, 16kHz and 32kHz were measured preoperatively and then 1 week, 1 month and 2 months postoperatively. Cochlear histopathology was examined at the conclusion of the study.

In the human study, a randomised controlled trial was conducted in a tertiary implant centre. Post-lingual, deaf adult CI candidates (n=30) were enrolled with preoperative audiometric thresholds of ≥ 80 dB at 125Hz and 250Hz, and ≥ 90 dB at 500Hz and 1000Hz. All subjects had failed a trial of hearing aids and had no contraindications to surgery. Subjects were randomised to either a control group, an oral steroid group receiving 1mg/kg/day of prednisolone up to a maximum dose of 60mg/day for 6 days prior to surgery, or a transtympanic steroid group receiving 0.5ml of 10mg/ml dexamethasone at 24 hours prior to surgery. Pure tone audiometry, Consonant Nucleus Consonant (CNC) word score, and AZ Bio sentence scores (in quiet and in noise in the implant only ear) were performed preoperatively and then at 1 week, 1 month, 3 months, 6 months and 12 months following implant activation. In addition, the pure tone average (PTA) and hearing preservation rate was calculated. All patients received the same electrode.

Results

At 1-week postoperative in the animal study, both groups receiving dexamethasone prior to implantation had smaller threshold shifts across all frequencies and this was significant at 32kHz ($p < 0.05$). There were no differences among the three groups in terms of electrode-related fibrosis. Spiral ganglion neuron (SGN) density was significantly higher in the group receiving steroids for 5 days, but only in the basal cochlear turn.

In the human study, subjects receiving transtympanic steroids experienced a significant decrease in the PTA over the 12-month period compared to the oral group and transtympanic steroid group, both of which showed an increase in the PTA ($F[4.85, 55.793] = 2.547, p = 0.04$). This effect was most pronounced in the first 3 months after implant switch on. In addition, the transtympanic steroid group showed improvement in the hearing preservation rate over the first 3 months compared to other groups, although this effect was not seen after 6 months. There were no significant differences in speech performance between the groups.

Conclusion

This study demonstrates the benefits of extended preoperative systemic steroids on hearing outcomes and SGN density in an animal model of CI surgery. In humans, preoperative transtympanic steroids appear to have a beneficial effect on reducing the hearing loss following CI during the first year following surgery, with the greatest effect seen within the first 3 months.

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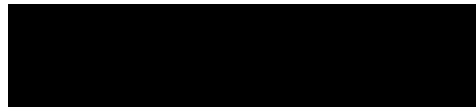
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Contents

Thesis Declaration	ii
Abstract	Error! Bookmark not defined.
Authorship Declaration	vii
List of figures	xi
List of tables	xii
Abbreviations	xiii
Acknowledgments	xiv
1. Introduction	1
1.1. Hearing preservation	3
1.2. Steroids and hearing effects	4
1.3. Mechanism of steroid activity in the inner ear	6
1.4. Route of administration.....	10
1.5. Side effects of steroids	15
1.6. Preoperative steroids and cochlear implantation – animal studies	18
1.6.1. Overview of published studies.....	18
1.6.2. Difficulties with current animal studies on the role of preoperative steroids and cochlear implantation.....	25
1.7. Preoperative steroids and cochlear implantation – human studies	27
1.7.1. Preoperative steroids – application to human cochlear implant surgery.....	33
1.7.2. Hearing loss after implantation – is this influenced by preoperative steroids?.....	35
1.8. Current gaps in the knowledge	38
1.8.1. Duration and method of steroid administration	39
1.8.2. Animal studies	39
1.8.3. Human studies and randomised controlled trials	40
2. Study hypothesis and aims	41
2.1. Hypothesis.....	41
2.2. Aims	41
3. Materials and methods	42
3.1. Animal study – materials and methods	42
3.1.1. Animals.....	42
3.1.2. Testing protocol.....	42
3.1.3. Theory and calculation	43

3.1.4.	Auditory testing	44
3.1.5.	Cochlear implant surgery.....	45
3.1.6.	Cochlear implant electrode	46
3.1.7.	Histology	47
3.2.	Human study – materials and methods.....	48
3.2.1.	Subject recruitment	47
3.2.2.	Treatment details	50
3.2.3.	Postoperative testing protocol.....	52
3.2.4.	Data analysis.....	53
4.	Results	54
4.1.	Animal study	54
4.1.1.	Auditory threshold results.....	54
4.1.2.	Histology results	55
4.1.3.	Discussion.....	60
4.1.4.	Conclusion	69
4.2.	Human study	70
4.2.1.	Patient flow.....	70
4.2.2.	Demographics and preoperative measures.....	71
4.2.3.	Treatment outcomes.....	76
4.2.4.	Pure tone audiometry and speech outcomes	76
4.2.5.	Discussion.....	79
4.2.6.	Conclusion	83
5.	General discussion	85
5.1.	Summary of results	85
5.2.	Overall limitations.....	86
5.3.	Systemic preoperative steroids in humans versus animals	88
5.4.	Intravenous versus intraperitoneal route	89
5.5.	Systemic versus transtympanic route	92
5.6.	Long-term results – can hearing loss after implantation be influenced by steroids?.....	93
5.7.	Steroid eluting electrodes	96
5.8.	Consensus statements on steroid use in cochlear implantation and current clinical implications	98
5.9.	Future directions.....	99
	Bibliography	101
	Appendix	114
	Ethics applications and supporting documentation	114

List of figures

Figure 3.1	Diagram of animal testing protocol	43
Figure 3.2	Greenwood function in the guinea pig	44
Figure 3.3	Guinea pig CI electrode [Source: MedEL Austria].....	46
Figure 3.4	Guinea pig CI electrode, close up with measurements	47
Figure 3.5	Diagram of human testing protocol	52
Figure 4.1	Typical section showing electrode tract within the scala tympani and surrounding fibrosis (haematoxylin and eosin stain)	56
Figure 4.2	8kHz threshold shift at 2 months versus basal SGN density at 2 months (cells/mm ²)	58
Figure 4.3	16kHz threshold shift at 2 months versus basal SGN density at 2 months (cells/mm ²).....	59
Figure 4.4	Patient flow diagram showing the recruitment pathway of patients and the final cohort included in the analysis.....	69
Figure 4.5	Mean pure tone thresholds at each frequency for the control, oral steroid and transtympanic groups over 12 months	76

List of tables

Table 1.1	List of studies where preoperative steroid administration is specifically mentioned in the methodology section	29
Table 4.1	Threshold shifts (from preoperative hearing level) across all three groups	54
Table 4.2	Histopathological results for all three animal steroid groups.....	56
Table 4.3	Electrode related fibrosis and SGN densities for all three groups	57
Table 4.4	Pearson correlation co-efficient values between SGN density (cells/mm ²) and threshold shifts at 1 week, 1 month and 2 months (in dB compared with control).....	59
Table 4.5	Mean IHC and OHC counts for each of the three groups at the basal, mid and apical turns of the cochlear	60
Table 4.6	Demographics of patients excluded from the study.....	73
Table 4.7	Demographics of patients included in the final cohort	74
Table 4.8	Preoperative mean hearing and speech discrimination scores for all three groups	75
Table 4.9	Mean PTA and hearing preservation rate in all three groups over all time points.....	77
Table 4.10	Mean word and sentence speech discrimination scores over all time points	79

Abbreviations

ABR	Auditory brain stem response
AF-PTA	All frequency pure tone average
CAP	Compound action potential
CI	Cochlear implant (ation)
CNC	Consonant Nucleus Consonant
EAS	Electroacoustic stimulation
ECAP	Electrically evoked compound action potential
ECOG	Electrocochleography
IHC	Inner hair cell
LF-PTA	Low frequency pure tone average
OHC	Outer hair cell
PTA	Pure tone average
RWM	Round window membrane
SD	Standard deviation
SGN	Spiral ganglion neuron

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1. Introduction

Cochlear implants (CI) are the most successful neural prosthesis to date (Wilson et al., 2008). From the very beginning of the CI concept with a single channel device (House et al., 1978), there are now an estimated 300,000 CI implanted to date since the first multichannel device was inserted in 1977 and 1978 (Clark et al., 1979; Hochmair, 1980). It is estimated the number of adults in Australia with partial or complete hearing loss is 10% of the population, or 2.1 million people (Access Economics, 2006). In addition, the risk of hearing loss increases with age, meaning that an ageing problem will only contribute further to this burden. In Australia, the number of individuals >65 years increased by 19% from 2008 to 2013 to reach 3.34 million people, or 14% of the population. In the USA, of the 16 million individuals aged >70 years with hearing loss, 150,000 are thought to fulfil the criteria for CI surgery (Lin et al., 2012).

Whilst CI technology has irrevocably changed the management of patients with severe to profound hearing loss, its widespread use and significant cost has led to its cost effectiveness coming under scrutiny. Despite this, unilateral CI in the context of bilateral severe to profound hearing loss are still considered to be cost effective (UK Cochlear Implant Study Group 2004; Palmer et al., 1999; Molinier et al., 2009).

Despite its cost effectiveness, the expense of CI technology combined with the current climate of increasingly scarce healthcare resources has led to a focus on candidacy. Several countries around the world have set in place candidacy guidelines to limit CI to those deemed most in need (National Institute for Health and Care Excellence (NICE)

Guidelines, 2009), whilst some have been less formulaic and more prescriptive by setting general guidelines for clinicians to follow (Western Australian Department of Health, 2013). It is therefore no surprise that the initial criteria of CI candidacy was limited to adults with severe to profound bilateral sensorineural hearing loss with poor speech discrimination who would clearly not benefit from a conventional hearing aid. This criterion has subsequently been questioned, especially since it has been shown that individuals with thresholds above 60dB HL have poor results with hearing aids (Ching et al., 1998; Hogan et al., 1998). Subsequently there has been a steady widening of the criteria to include implant patients with more residual hearing (Fraysse et al., 1998). There has also been a growing awareness of the impact and disability of “partial deafness”. In the early beginnings of CI technology, these were not considered severe enough to warrant implantation (Skarzynski et al., 2002; Kiefer et al., 2005; Skarzynski 2007; Prentiss et al., 2010).

Over the last decade, there has been an increasing focus on the concept of hearing preservation, or the ability to preserve hearing following CI surgery. In its most acute form, patients with ski-slope hearing loss were found to benefit from electrical stimulation despite having preserved low frequency hearing. Such patients with incomplete deafness could utilise both their existing acoustic low frequency hearing in combination with the high frequency electrical stimulation from a CI. The concept of electroacoustic stimulation (EAS) led to an expansion of research in the field of hearing preservation (von Ilberg et al., 1999). This concept has been further expanded and

studied by several notable authors (Gantz et al., 2004; Gstöttner et al., 2009; Kiefer et al., 2004; Lenarz et al., 2006).

1.1. Hearing preservation

Residual hearing is important because it has been shown to be important for speech and music perception (Gfeller et al., 2006; Büchner et al., 2009) as well as sound localisation and hearing in noise (Wilson et al., 2003). Carlson in a retrospective review of 126 implant recipients found that patients with measurable residual hearing in the implant-only ear performed better in speech testing (Carlson et al., 2015).

The residual hearing which is of particular importance and is currently the focus of most residual hearing research is low frequency hearing. It is well known that in most cases of progressive sensorineural hearing loss, the low frequencies are often preserved until late. This is primarily due to the resilience of the apical region of the cochlear hair cells, resulting in preservation of function at low frequency or apical region of the cochlear (Kopke et al., 1999). Low frequency hearing is important for listening in complex environments and for carrying the fundamental frequency of voice (Gifford et al., 2010; Zhang et al., 2010).

In classic electric acoustic stimulation where the degree of residual hearing is high, attempts have been made to create shorter and less traumatic electrodes in order to minimise trauma and preserve as much residual hearing as possible. This has led to a variety of reduced length electrodes which have been demonstrated in clinical practice (Gstöttner et al., 2009; Skarzynski et al., 2012; Gantz et al., 2004; Lenarz et al., 2006).

However, significantly longer length electrodes of up to 31.5mm have been implanted and showed hearing preservation by several different groups (Baumgartner et al., 2007; Kiefer et al., 2004; Usami et al., 2011; Helbig et al., 2011; Tamir et al., 2012). Hearing preservation limited to adults but has also been demonstrated in children (Kuthubutheen et al., 2012; Skarzynski et al., 2007). The ability to preserve hearing is particularly important in children where there is a very high likelihood of these patients requiring reimplantation at some point during their life. The ability to preserve hearing during cochlear *re-implantation* has also been demonstrated in children (Jayawardena et al., 2012). From these studies, it is clear that being able to preserve residual hearing is not only possible in a wide variety of situations but rather almost now *expected* in contemporary CI surgery. The focus has therefore been on ways to maximise and preserve this residual hearing by as many methods as possible. These have included the use of “soft surgery” (Lenhardt et al., 1993), atraumatic electrode design (as mentioned above), altering the speed of insertion (Kontorinis et al., 2011), and pharmacological methods. A wide variety of pharmacological methods have been studied including neurotrophins (Miller et al., 1994; Havenith et al., 2011), gene therapy via adeno-associated virus (Lalwani et al., 1996), and novel anti-inflammatory drugs (Meyer et al., 2012). These methods which is of particular interest to this thesis is the use of steroids.

1.2. Steroids and hearing effects

Steroids are well recognised to have protective effects on the inner ear (Takemura et al., 2004). These effects have led to the study of steroids in a wide variety of conditions that

are thought to have an inflammatory component to their pathophysiology. These conditions include the use of steroids for the treatment of Meniere's disease, vestibular labyrinthitis, sudden sensorineural hearing loss, noise-induced hearing loss and autoimmune inner ear disease, ototoxicity and prior to stapes surgery (Hu and Parnes, 2009).

Pietro-Casani et al. compared three intratympanic injections once every 3 days of 4mg/ml dexamethasone compared to gentamicin for the treatment of Meniere's disease. They found a better rate of hearing preservation but poor control of vertigo attacks in the group receiving steroid (Pietro-Casani et al., 2012). Barrs et al., found that multiple courses of transtympanic dexamethasone delivered through a ventilation tube resulted in control of vertigo attacks in 47% of patients with Meniere's disease (Barrs et al., 2004). Silverstein et al., first described the use of steroids transtympanically for the treatment of sudden sensorineural hearing loss (Silverstein et al., 1996). In a systematic review of the use of steroids for the treatment of sudden sensorineural hearing loss, the authors concluded that intratympanic steroids offered an advantage as a secondary treatment option when systemic steroids had failed (Garavello et al., 2012). In another study to assess the role of steroids in cisplatin-induced ototoxicity, dexamethasone was found to reduce hearing loss in a guinea pig model of ototoxicity. Specifically, when intratympanic dexamethasone was given bilaterally the day before and on the day of cisplatin administration with a dose of up to 24mg/ml, hearing loss at 8kHz was reduced (Murphy et al., 2011). In another study, aminoglycoside-induced ototoxicity was used to assess the effectiveness of dexamethasone. Kanamycin-induced hair cell loss and

hearing loss was reduced by intracochlear dexamethasone infiltration at 1ng/ml for 28 days prior and for 14 days pre- and post-kanamycin. Surprisingly, a higher dose of 10ng/ml resulted in hair cell protection but not hearing preservation, suggesting the possibility of direct ototoxicity. Dexamethasone treatment after kanamycin was not as effective, suggesting that pre-treatment of the inner ear is more important than post-treatment alone (Himeno et al., 2002).

Ariyasu et al. found beneficial effects of treating patients with acute vestibular neuronitis with systemic methylprednisolone in a double-blinded placebo controlled study (Ariyasu et al., 1990). Similarly, Strupp et al. found systemic methylprednisolone was superior to valacyclovir in restoration of vestibular function at 1 year following acute vestibular neuronitis (Strupp et al., 2004).

Steroids have also been shown to be beneficial in Cogan's syndrome, a type of autoimmune inner ear disease (Haynes et al., 1981). Steroids have also been shown to have a protective effect against noise-induced hearing loss (Henry et al., 1992). In an operative study to assess the use of prednisolone prior to stapes surgery for otosclerosis, no beneficial effects were found (Riechelmann et al., 2000).

1.3. Mechanism of steroid activity in the inner ear

Considerable work has already been undertaken to elucidate the mechanism of action of steroids in the inner ear. In its most basic form, steroids act in all mammalian cells in the same manner through both glucocorticoid and mineralocorticoid receptors (Ballard et al., 1974). There are two phases of time in which steroids interact with individual cells.

The first occurs rapidly in seconds to minutes in a so-called “non-genomic” signalling pathway that involves the interaction of steroids, both glucocorticoid and mineralocorticoids, with receptors at the cell membrane surface (Lösel et al., 2003; Gametchu et al., 1993; Boldyreff et al., 2003). Non-genomic effects of steroids on the potassium ion channel in the stria vascularis have also been demonstrated (Lee et al., 2002). The second phase occurs over a much longer period of time (typically several hours) and involve steroids traversing the cell membrane to bind with intracellular receptors which then migrate to the nucleus. This then affects the transcription of proteins either by upregulation or suppression, leading to widespread cellular effects (Löwenburg et al., 2008). Clearly both temporal effects are important and have led to the development of selective glucocorticoid receptor agonists which selectively target receptor ligands and result in specific cellular effects with fewer side effects (van der Laan et al., 2008).

Steroid receptors are widely distributed in the tissues of the inner ear in both hearing and vestibular end organs, as observed in multiple animal studies (Rarey et al., 1996; ten Cate et al., 1993; Pitovski et al., 1994; Erichsen et al., 1996). They have also been found in surrounding structures such as the spiral ligament and stria vascularis, the latter being important in potassium ion homeostasis (Zuo et al., 1995). Their mechanism of action can be divided into mineralocorticoid effects via their action on sodium and critical potassium ion channels to affect endolymphatic fluid potential, glucocorticoid effects that affect sodium ion resorption in the semi-circular canals, as well as immunological and anti-inflammatory pathways (Pondugula et al., 2006). These effects, however, partly

overlap with both mineralocorticoid and glucocorticoid receptor activity that is required to work in tandem to mediate the effects of glucocorticoids, since glucocorticoids are also able to bind to the mineralocorticoid receptor (Trune et al., 2006; Trune et al., 2007). Steroids may also affect aquaporins in the inner ear (Fukushima et al., 2004).

There is widespread evidence for genomic effects of steroids in the inner ear. Maeda et al. demonstrated that transtympanic dexamethasone injected into the middle ear of mice resulted in up-regulation of the Fkbp5 protein (Maeda et al., 2010). Fkbp5 expression is widely distributed throughout the inner ear (less so in inner hair cells, IHCs) and occurs between 3 and 24 hours after dexamethasone exposure. Its effects are equally widespread and include immunosuppression (through FK506), apoptosis inhibition and even sound transduction in the Organ of Corti function (Maeda et al., 2010; Romano et al., 2010; Rifai et al., 2006). Hoang et al. demonstrated that dexamethasone applied to organ of Corti explants resulted in upregulation of nuclear-factor kappa B protein (Hoang et al., 2009). This protein acts as a hair cell protectant by inhibiting tumour necrosis factor α (TNF- α)-induced apoptosis (Dinh et al., 2011). TNF- α is one of the common mediators of inner ear cellular damage and apoptosis that can be induced by multiple insults such as ototoxicity, noise-induced hearing loss or infection (Haake et al., 2009).

Steroids have also been shown to increase blood flow to the inner ear. This may be beneficial by preventing the reduction in blood flow observed in patients receiving CI (Nakashima et al., 2002). Using laser Doppler vibrometry, Shirwany et al., demonstrated

that dexamethasone administered intratympanically to the round window resulted in a mean increase in blood flow of nearly 30% within half a minute of application and lasted for up to 1 hour after recording had ended (Shirwany et al., 1998). This implies that non-genomic effects occur at multiple areas within the cochlea.

In addition to reducing the inflammatory response, corticosteroids and particularly glucocorticoids, also act as suppressants of the immune system. This occurs through a variety of mechanisms, including effects on cellular immunity by inducing the apoptosis of thymocytes through the caspase pathway which has been shown to occur with dexamethasone (Cifone et al., 1999). Glucocorticoids also suppress the Th1 cellular immune response and promote upregulation of the Th2 humoral immune response. By affecting the activity of interleukin-12, glucocorticoids reduce the activity of natural killer cells and CD8 lymphocytes responsible for cytotoxicity (Franchimont et al., 2000). Other indirect evidence of these effects can be seen in the effect of glucocorticosteroids on other organs in the body. These include the retina, where it has been shown to reduce neovascularisation and macrophage invasion (Ishibashi et al., 1985), the liver where there is a reduction in hepatic fibrosis (Dufour et al., 1997), and cardiac pacemaker leads where steroids eluting electrodes produce a reduction in impedance and threshold (Anderson et al., 1991; Mond et al., 1996).

Together with the genomic effects, this implies that short exposure to steroids alone does not predict poor response as there are ongoing downstream effects which can only be assessed by examining the genomic response of the inner ear target cells.

1.4. Route of administration

While there is evidence for inner ear steroid effects both *in vivo* and *in-vitro*, there remains considerable disagreement in the literature as to which method of delivery is superior. Given the location of the inner ear and its relative inaccessibility, the two main methods which have been adopted are local drug delivery and systemic administration. Local drug delivery techniques include transtympanic injections, round window placement of a drug eluting device or reservoir, intracochlear injection, or drug eluting methods. Systemic administration can include intravenous, oral or intraperitoneal routes. Given the wide variety of options available, it can be difficult to compare the outcomes due to the different pharmacokinetics of the various delivery methods. In the transtympanic route, the primary mode of steroid entry into the inner ear is through the round window (Plontke et al., 2007). In the systemic route, the steroid crosses the blood-perilymph barrier which has a similar properties to the blood/brain barrier in terms of ion transport mechanisms and permeability to drugs (Sterkers et al., 1987; Tobita et al., 2002; Juhn et al., 2001).

In one of the earliest papers to address this issue, Parnes et al. used an animal model to investigate three steroids (hydrocortisone, methylprednisolone and dexamethasone) and three modes of drug delivery (oral, intravenous and intratympanic) (Parnes et al., 1999). For each drug, a high and low systemic dose was chosen as well as an intratympanic dose. Drug levels were then sampled in the blood, CSF, perilymph, and endolymph. For dexamethasone, only the high dose IV (8mg/kg compared to 0.2mg/kg low dose) resulted in detectable levels in the perilymph. The intratympanic dexamethasone route

(0.11ml of 4mg/ml) resulted in significantly higher inner ear concentrations. For hydrocortisone and methylprednisolone, endogenous plasma levels were detected and oral and IV levels were detected after their relevant administration in a dose dependent fashion. Intratympanic levels were significantly higher than systemic routes as in dexamethasone. The Parnes et al. study showed that high dose intravenous administration results in measurable inner ear levels, although the transtympanic route delivers much higher concentrations (Parnes et al., 1999)

A later study performed by Bird et al. reported similar results in humans (Bird et al., 2011). To our knowledge this is the only study in which perilymph concentrations were measured in humans. The authors compared intratympanic (4mg) versus intravenous dexamethasone (0.17mg/kg). Both perilymph and plasma concentrations were measured up to 90 minutes post-dosing. Intratympanic dexamethasone resulted in an 88-fold (260-fold after dose correction) increase in perilymph drug concentrations compared to the intravenous route. The plasma concentration was five times lower for the intratympanic route compared to the systemic route. However, significant variability was noted in the concentrations following the perilymph method. This variability is the major limitation of the transtympanic route.

The time course for dexamethasone distribution and uptake in the inner ear has also been studied for more extended periods. Using an animal model, Hargunani et al. examined the pharmacokinetics of intratympanic dexamethasone from its prodrug sodium phosphate formulation to its free form (Hargunani et al., 2006). Based on

immunohistochemical staining, the steroid was at its highest concentration between 30 to 60 minutes post-injection, while complete clearance of the drug was seen at 24 hours. The predominant location of dexamethasone uptake was in the Organ of Corti and spiral ligament. Critical uptake sites relevant for CI were in the region of the spiral ganglion neuron (SGN), as well as at the apex of the cochlea. The finding of dexamethasone uptake being correlated with glucocorticoid receptor staining was encouraging, as this suggests delivery to the correct site of action.

In an earlier study, Chandrasekhar et al. examined the pharmacokinetics of intratympanic dexamethasone in animals and found a similar pattern of higher perilymph levels compared to the systemic route (Chandrasekar et al., 2000). In addition, the intratympanic route resulted in peak inner ear concentrations at 1 hour after instillation, a finding similar to previous studies.

Several potential factors may affect the transtympanic route. Assuming the round window is intact at the time of drug infiltration, these factors include loss of drug through the Eustachian tube (Salt et al., 2011), the permeability of the round window membrane (RWM) (Hahn et al., 2006), the presence of middle ear mucosal disease, a round window mucosal fold (Alzamil et al., 2000), and air bubbles adjacent to the RWM. The ciliated respiratory epithelium of the middle ear mucosa also contributes to the clearance (Hentzer et al., 1984). Mikulec et al. showed that benzylalcohol increases RWM permeability by a factor of up to 5-fold, increases osmolality to 620 milliosmoles by 3-fold, and surprisingly drying the RWM by up to a factor of 15-fold (Mikulec et al.,

2008). The study also noted that triamcinolone, a steroid commonly used in CI surgery, is up to 5µm in size and therefore too large to cross the RWM.

Based upon pharmacokinetic studies, the primary determinant of steroid uptake via the transtympanic route for a given concentration of steroid is duration within the middle ear (Salt et al., 2008). Apart from optimising the route and time of exposure, another factor which should not be overlooked is the non-uniform concentration within the inner ear. It is well known there is a decreasing basal to apical concentration of steroids which is based upon inner ear fluid pharmacokinetics and distribution mechanics. In a study using a single shot of intratympanic dexamethasone in guinea pigs, the basal to apical concentration gradient was approximately 17,000-fold (Plonke et al., 2008). The authors also estimated the clearance of dexamethasone ranged from 65 minutes to more than 8 hours, depending upon the permeability of the round window. By comparison, the clearance of intratympanic methylprednisolone is just over 2 hours (Plontke et al., 2008; Mikulec et al., 2009) and the half-life of systemic dexamethasone in plasma is ranges from 2 to 5 hours (Czock et al., 2005). It should be noted that other routes of steroid absorption have not been considered. In animals, the thin bone of the otic capsule can result in absorption of steroid at the apex when applied transtympanically (Salt et al., 2011). This can potentially limit the applicability of animal transtympanic results to humans, where the thickness of the otic capsule is significantly greater (Mikulec et al., 2009). The oval window may also be the site of entry (Salt et al, 2008, 2009, 2012).

In an attempt to overcome some of the limitations of intratympanic drug administration, the intracochlear delivery route has also been studied. This offers unparalleled direct access to the inner ear through an opening in the bony otic capsule either via the round window or through a cochleostomy. Such routes are usually limited in human subjects to the time of surgery just prior to CI electrode insertion when there is already a deliberate opening into the inner ear. In an animal study, dexamethasone phosphate (10mg/ml) was injected through the round window or into a cochleostomy (Hahn et al., 2012). This resulted in a 10-fold higher concentration than could be achieved by up to 3 hours of intratympanic injections. The variability typically seen with intratympanic injections was reduced and perilymphatic drug concentrations were detectable for almost 4 hours by apical perilymph sampling, as compared to just 1 hour for the intratympanic route. The decreasing base to apex concentration gradient usually seen with intratympanic injections was also less apparent. Middle ear catheters have also been studied for ambulatory delivery of steroids (Plontke et al., 2009).

Another method to overcome the limitations of the transtympanic route has been the use of gel formulations instilled into the inner ear in order to prolong the drug. By utilising a poloxamer 407 hydrogel which is a liquid at room temperature and gel at body temperature, dexamethasone can be coupled to the gel and provide sustained delivery from 10 days up to 3 months (Wang et al., 2009; Wang et al., 2011a, b). No major side effects were reported, although a mild inflammatory response in the middle ear was noted (Piu et al., 2011) as well as temporary conductive hearing loss (Salt et al., 2011).

Due to the disadvantages of the transtympanic route and in spite of its advantages, the systemic steroid delivery route is still of interest. The primary advantage of the systemic route, and in particular of the intravenous route, is the ease of administration especially around the time of surgery when intravenous access is readily available. In an animal study, intravenous prednisolone at 100mg/kg was administered and tissues from the temporal bone, liver, brain and serum were harvested at regular intervals from 30 minutes to 8 hours after dosing (Tobita et al., 2002). The highest concentration of prednisolone was observed at 30 minutes in serum and liver, with the cochlear tissue reaching its peak concentration at 1 hour after dosing. The levels in the serum and liver declined rapidly after peaking but the cochlear tissues retained their peak concentration after 4 hours. This suggests that in contrast to the transtympanic route, there is a more sustained delivery of steroids from the periphery where there has been equilibration. This is in contrast to the Parnes et al., study, which measured the steroid levels in the fluid spaces of the inner ear rather than the tissues (Parnes et al., 1999).

1.5. Side effects of steroids

Hu and Parnes in their review of the literature from 1966 to 2009 identified considerable heterogeneity in studies that examined the role of intratympanic steroids for the treatment of Meniere's disease and sudden sensorineural hearing loss (Hu and Parnes, 2009). This highlights the difficulty in comparing results and in determining the incidence of side effects.

Several complications with the use of steroids have been reported. When given via the transtympanic route, local complications have arisen due to infiltration of a fluid substance through an intact tympanic membrane (Hamid et al., 2008). When performed under local anaesthesia, pain and vertigo from caloric effects is probably the most common side effect (Parnes et al., 1999; Lefebvre et al., 2002; Kopke et al., 2001; Ho et al., 2004; Herr et al., 2005). The chorda tympani nerve which lies just medial to the posterior edge of the tympanic membrane may be susceptible to injury, producing dysgeusia (Herr et al., 2005). Because transtympanic injections occur through an intact tympanic membrane, perforations have been reported (Slattery et al., 2005; Herr et al., 2005) and can lead to infections which may be acute or chronic (Parnes et al., 1999; Herr et al., 2005). Long-term tympanic membrane perforations have not been attributed to steroid use (Parnes et al., 1999). Systemic hyperglycemia has been reported although due to the extremely low systemic absorption from transtympanic injection, this is considered very rare (Gallegos-Constantino et al., 2011). Some authors have reported ototoxic effects of locally applied steroids in animal studies, although this is uncommon. Spandow et al. reported mid to high frequency hearing loss after the application of 2% hydrocortisone to the round window in rats, suggesting basal cochlear turn ototoxicity (Spandow et al., 1989). The tympanic membrane perforation was also observed to be persistent. In another study, pneumococcal meningitis-induced hearing loss in animals treated with intratympanic betamethasone developed worse low frequency hearing, despite better SGN counts (Worsøe et al., 2010). Surprisingly the steroid treated group developed significant tympanic membrane fibrosis which was not observed in the saline

treated groups. The authors surmised this may be a species-related effect. In a later study, the effect of topical dexamethasone and hydrocortisone on RWM histology was compared (Nordang et al., 2003). Interestingly the hydrocortisone treated group developed RWM inflammatory changes, whilst the dexamethasone group did not. Arriaga et al., reported a 20% reduction in audiometric and speech perception in a group receiving intratympanic steroids for endolymphatic hydrops, although with no control group it is difficult to separate this effect from the natural course of the condition (Arriaga et al., 1998). Despite this, the risk profile of transtympanic injections are considered low, especially given the ubiquity of topical corticosteroid use for perforated tympanic membranes and ventilation tubes.

The side effects of systemic steroids are more common than for local steroid administration and have been well documented. The short-term side effects of systemic steroids are most applicable to the inner ear, unless long-term steroids are used for autoimmune inner ear disease. Cushingoid appearances typically occur after 2 months of treatment (Curtis et al., 2006). The most feared complication is femoral head necrosis, the risk of which increases with dose and duration of treatment but can still occur with shorter courses of therapy (Weinstein et al., 2012). Other potential short-term side effects include diabetes, euphoria, hypertension, increased appetite, fluid retention and gastrointestinal upset (Moghadam-Kia et al., 2010). Hyperglycemia is a dose-dependent phenomenon and occurs within several hours of commencing systemic dexamethasone (Schneider et al., 1998). At 60mg/kg, the odds ratio of developing hyperglycemia is approximately 3-fold (Gurwitx et al., 1994). Psychiatric disturbances including mood

swings and sleep disturbance have also been reported, especially in courses of treatment lasting more than 1 week (Turner et al., 1993). Hypertension and other cardiovascular disturbances including arrhythmias have been reported with doses larger than 7.5mg (Wei et al., 2004). Adrenal suppression has been typically associated with long-term use of steroids, but has also been reported in courses as short as 5 days with 25mg prednisolone (Henzen et al., 2000). The incidence of gastrointestinal ulcers is increased with systemic steroids in the setting of non-steroidal anti-inflammatory use where it should be cautioned (Saag et al., 2012).

1.6. Preoperative steroids and cochlear implantation – animal studies

1.6.1. Overview of published studies

The protective inner ear effect of steroids lends itself well to being used in CI. Most of the published literature on the use of preoperative steroids in CI have been on animal models, with considerably fewer studies in humans. Studies on the use of steroids to reduce hearing loss have primarily focussed on the administration of steroids just prior to surgery. The longest duration of steroid application in a published study is 6 hours prior to surgery (Quesnel et al., 2011). The most common animal model of CI is the guinea pig. Apart from being readily available, easy to maintain in animal laboratories and docile in nature, the temporal bone and inner ear of the guinea pig are well suited to experimentation. There are many similarities in hearing between the guinea pig and human (Mohammadpour et al., 2011). The bulla of the temporal bone is easily accessible and once opened, the cochlear is immediately visible with the round window

orientated in the horizontal plane (Sanli et al., 2009; Wysocki et al., 2005a). The middle ear space is air-filled and limited laterally by a tympanic membrane which connects to the inner ear through an ossicular chain, ending at a vertically oriented oval window (Sanli et al., 2009). Detailed microscopic examination of the cochlear allow the design of appropriately sized implants that are sub-millimeter in the basal turn of the cochlear (Wysocki et al., 2005a). There are, however, more spirals in the guinea pig with 3.5 to 3.75 turns compared to 2.5 in the human (Wysocki et al., 2005b).

An animal model of CI was used by Ye et al. to assess the effect of triamcinolone on hearing and electrophysiology in response to cochleostomy trauma (Ye et al., 2007). Two groups of guinea pigs were used with the contralateral ears as controls. In the first group, a low dose of triamcinolone (0.2mg) was applied to the round window by gelfoam. In the second group a higher dose (0.12mg) of the same steroid in suspension was applied via a direct intracochlear injection through a cochleostomy which was then closed. Acoustic, click-evoked compound action potentials (CAP) were recorded from a round window electrode for up to 4 weeks. Growth functions of the CAP were recorded for increasing sound intensities to determine the maximum amplitudes (IHC and cochlear nerve function) and threshold (outer hair cell (OHC) function). In the low dose group, greater maximal amplitude was observed in the ears receiving steroid, with no changes in threshold. This suggests the existence of an inner ear protective effect following trauma. In the high dose intracochlear group, ears that received steroids showed a lower CAP threshold shift within a few days until a return to the preoperative

level by 1 month. The largest threshold shifts observed in all groups were for the high frequencies, corresponding to the location of the basal turn of the cochleostomy.

Huang et al. used guinea pig and cat models to compare the use of intracochlear dexamethasone and triamcinolone just prior to implantation of 4mm and 6mm electrodes, respectively, for up to 5 months (Huang et al., 2007). The cats were stimulated electrically, whilst ABR and electrical impedances were recorded in both. Cats but not guinea pigs receiving triamcinolone had lower impedances for 2 months before rising to match the control groups, suggesting a species-specific effect. In the guinea pigs, more fibrosis and higher impedances were seen in the steroid treated animals but there was no correlation between impedance and tissue response. Interestingly in the dexamethasone treated group, the apical turn SGN density was lower but this was attributed to aspiration and injection of perilymph rather than the steroid itself.

Using a guinea pig model, Eshraghi et al. employed a different model of CI surgery comprising of electrode insertion and removal of a 3mm x 0.14mm diameter steel electrode via a cochleostomy (Eshraghi et al., 2007). Three groups were compared – a control group where the cochleostomy was closed, a second group that received artificial perilymph post-electrode trauma, and a third group receiving postoperative 100ug/ml dexamethasone for 8 days via an osmotic pump inserted into the cochleostomy. ABR measurements were performed for 30 days. The animals receiving the steroid had better hearing after 1 month compared to other groups which continued to have elevated

thresholds. In a second publication by the same research group, a similar study was conducted using dexamethasone base rather than the prodrug (dexamethasone sodium phosphate). Hair cell counts as well as ABRs were also measured (Vivero et al., 2008). The study found that the dexamethasone treated group had similar levels of hearing compared to the control group, indicating a protective effect of postoperative steroids for up to 1 month. There was also greater preservation of outer and IHC counts compared to the groups which received electrode trauma but no steroids. The greatest level of hearing preservation was primarily in the lower frequencies. In a third study by the group, in addition to examining the effects of dexamethasone as in the previous two studies, organ of Corti explants were treated with TNF- α to assess whether dexamethasone acts to prevent cell death due to TNF- α -induced apoptosis (Van De Water et al., 2010). This study found that dexamethasone resulted in less hair cell death. Real time PCR analysis of the steroid treated explants revealed upregulation of the known anti-apoptotic genes *Bcl-2* and *Bcl-xl* and downregulation of genes known to promote apoptosis (*Bax* and *TNFR-1*). This work supports the findings of Hoang et al. that showed activation of NFkB was an intermediary step (Hoang et al., 2009).

In a series of papers from a group working in Melbourne (James et al., 2008; Maini et al., 2009; Chang et al., 2009; Eastwood et al., 2010; Connolly et al., 2011; Souter et al., 2012), the focus has been on the role of preoperative steroids rather than on immediate postoperative steroids. The first paper by James et al., describes a guinea pig model of CI which differs from the Miami group in that the electrode was implanted and left in situ to an insertion depth of 2.25mm (James et al., 2008). Dexamethasone at a concentration of

2% was used to soak a hyaluronic acid/carboxymethylcellulose bead (Seprapak). This was found to be the only drug carrier to result in measurable levels of steroid for up to 24 hours. The steroid depot was placed against the round window 30 minutes prior to surgery. From 1 hour post-implantation to 4 weeks post-surgery, the dexamethasone treated group had lower threshold shifts at 32kHz, the basal region of the electrode. In a select group of animals, the steroid treated group had no foreign body multinucleate giant cell reactions around the electrode and had a lesser histiocyte response, thus contrasting with the Huang et al.'s study (Huang et al., 2007). Hair cell or SGN counts were not assessed. These results suggest there is both an immediate as well as a delayed effect of dexamethasone, in keeping with the known pharmacodynamics of the drug.

Maini et al. expanded upon the results of the previous study by examining the auditory thresholds to 3 months as well as the SGN counts, which had not previously been assessed (Maini et al., 2009). In the dexamethasone treated animals, the hearing loss reduction at 32kHz was persistent till 3 months. At frequencies apical to the site of implantation (2, 8 and 16kHz), these threshold elevations had resolved to baseline. Basal SGN densities were significantly higher in the steroid treated group compared to the control group, although both groups had lower counts over time.

Chang et al. built further upon these results by increasing the time of application and the concentration of dexamethasone to determine whether these factors were important (Chang et al., 2009). As in the study by James et al., Seprapak was soaked in 2% dexamethasone but additionally in 20% dexamethasone (James et al., 2008). The bead

was then applied to the round window at 30 mins but also at 1 hour and 2 hours before electrode insertion. The insertion length was shorter at 1.75mm. Thresholds were no longer measure at 1-hour post-implantation due to their natural recovery as seen previously. By increasing the application time, the threshold shift at the basal turn decreased accordingly. At 8kHz and 16kHz, an application time of 2 hours was required to achieve preservation of hearing. A 30-minute application of 20% concentration was equivalent to a 2-hour application at 2% concentration. Histological evaluation was performed in approximately one third of animals, but no conclusions could be drawn from the results.

Eastwood et al. sought to separate the trauma due to the electrode insertion at the basal turn from the local application of steroids (Eastwood et al., 2010). This was performed by a second turn cochleostomy and insertion of the electrode until resistance, although the depth of insertion was not recorded. Similar time points and concentrations were used in Chang et al.'s study (Chang et al., 2009). Longer application times and higher concentrations were again associated with better hearing protection. Despite the second turn implant insertion, the basal turn high frequency region was still protected. More significant trauma and fibrosis was seen in the second turn but once again, the histological appearance did not correlate with the use of steroids.

After demonstrating that local steroid application at the round window is beneficial, the Melbourne group proceeded to investigate the role of preoperative intravenous dexamethasone (Connolly et al., 2011). The two concentrations used were 0.2mg/kg

(low dose) and 2mg/kg (high dose), given 1 hour prior to surgery (Connolly et al., 2011). No effect was seen for the low dose treated group, but a reduction in threshold shift was observed at the higher dose. This affected the high frequencies at 1 week and across a broader range of lower frequencies across all time points to 4 weeks. Investigation of thresholds in the unimplanted ear found that 2mg/kg of dexamethasone given systemically was not ototoxic. Histopathology revealed that the high dose treatment group had less variable fibrotic reaction; however, once again there were no differences in SGN densities. As in the study by Maini et al., this was not expected until at least 3 months (Maini et al., 2009).

The Melbourne group then examined the role of systemic immunity in the cochlear by priming guinea pigs with the sterile antigen, Keyhole-Limpet Hemocyanin (Souter et al., 2012). This was to determine if the resultant activation of systemic leucocytes would increase the hearing loss associated with CI and whether systemic dexamethasone could reduce this effect. The same animal model of CI was again used. A broader range of frequencies was found to be elevated in the primed animals, even beyond the site of implantation at 2kHz. Dexamethasone (20%) applied locally to the round window reduced this threshold shift, but less so at other frequencies. The primed animals also demonstrated a greater degree of fibrosis.

In a study by a French group, Quesnel et al. examined the role of preoperative systemic methylprednisolone in a guinea pig model that was similar to that used by the Melbourne group (Quesnel et al., 2011). An electrode was inserted 3mm into the basal turn of the

cochlea. ABR thresholds using click stimuli were used and up until 3 weeks post-surgery. Intramuscular methylprednisolone (SOLU-MEDROL 20mg/2ml) was used at a dose of 2mg/kg and was administered 6 hours and 2 hours prior to surgery, then 4 hours post-surgery. A labyrinthectomy of the contralateral ear was performed. The click ABR thresholds were significantly lower in the steroid treated group at all time points up to 3 weeks post-surgery. Histopathology was not performed but CT scans confirmed correct positioning of the implant within the basal turn.

1.6.2. Difficulties with current animal studies on the role of preoperative steroids and cochlear implantation

Whilst the above studies offer a detailed insight into the potential use of steroids in CI, there remain several issues which have yet to be addressed. In a study by Ye et al., whilst there was a benefit from steroid (triamcinolone) in ameliorating the hearing loss post-cochleostomy, no electrode was inserted (Ye et al., 2007). However, Ye et al. did measure the electrophysiological parameters that have been lacking in many other studies. The study by Huang et al. was comprehensive in its scope and assessment and utilised longer electrode lengths (Huang et al., 2007). However, the period of steroid application was just prior to electrode insertion. In the work by Eshraghi et al., the insertion and removal model of electrode trauma is arguably less applicable to clinical practice where CI electrodes are often left in situ for many years (Eshraghi et al., 2007). Although this model is able to assess the immediate trauma associated with cochleostomy and electrode insertion, it excludes the inevitable tissue response to an implanted electrode. The use of an osmotic pump into the cochleostomy site adds

complexity, can only be applied to situations where there is no electrode in situ, and potentially leaves the otic capsule open.

The studies from the Melbourne group offer a well-described, systematic approach to studying the role of preoperative as opposed to postoperative steroids (O’Leary et al., 2013). However, it could be argued the model has some disadvantages. The ABR thresholds were tested in a sound-field with the contralateral ear plugged. This may not have reduced inter-aural attenuation compared to the use of inset earphones. The electrode insertion depths of 1.75mm and 2.25mm are considerably shorter than depths used in other studies, which arguably may not reflect the current practice in humans to use relatively longer electrodes. The use of a round window depot of steroid helps to direct the drug more closely to the site of activity in the basal turn, but requires preparation and surgical exposure. When testing longer periods of preoperative steroid application, this can become problematic because the subject or animal must remain under general anaesthesia. Round window depot applications also preclude the use of the round window as an option for electrode insertion. Histological examination was also performed in only a select group of animals, thus potentially under powering the study and introducing bias.

There were also some limitations in the studies that examined the role of preoperative systemic steroids. In the study by Quesnel et al. demonstrating the benefit of at least 6 hours of preoperative systemic steroids, the authors could not distinguish the effects from postoperative steroids (Quesnel et al., 2011). Frequency specific analysis was not

available, nor the histopathology results for long-term effects of hair cell and SGN counts. In the study by Connolly et al., preoperative systemic steroids were only given for 1 hour prior to surgery and histological data for 1 month only was available (Connolly et al., 2011).

1.7. Preoperative steroids and cochlear implantation – human studies

Nineteen English language articles were found in which specific details of preoperative steroid administration were documented as part of the clinical protocol to study hearing preservation outcomes (Table 1.1). These studies varied widely in terms of the type of steroid used, dosages, concentration, and time of application before electrode insertion. The three most common types of steroids used were triamcinolone, methylprednisolone and dexamethasone. The two common time points for administration were at induction and just prior to opening of the membranous labyrinth, after which electrode insertion proceeded immediately. The duration of steroid exposure was not always specifically documented and where mentioned, varied from several minutes to more than 1 hour. The large variation in methodology and route of administration (in many cases a combination of different routes was used) makes it difficult to draw any firm conclusions from these studies regarding the benefits of preoperative steroids. A recent meta-analysis by Santa Maria et al. included several of the studies presented in Table 1.1 and concluded there was no association between preoperative steroid use and hearing preservation outcomes (Santa Maria et al., 2014). To date, only two prospective trials in humans have been published where the use of preoperative steroids was the main focus

of the study. The first showed that steroids reduced hearing loss (Enticott et al., 2011), while the second showed a reduction in vestibular symptoms (Rajan et al., 2012). In another study designed primarily to assess surgical technique, the highest published dose of preoperative intravenous steroids was used preoperatively (Keifer et al., 2004).

Table 1.1 List of studies where preoperative steroid administration is specifically mentioned in the methodology section

Author	Steroid(s) used	Dose / concentration	Route
Arnoldner et al., 2011	Triamcinolone	See note regarding dose of Volon A	Intratympanically after cochleostomy and before opening of endosteum, or just before RWM opening
Brown et al., 2010	Dexamethasone	0.25mg/kg	Intravenously on induction
Bruce et al., 2011	Triamcinolone	40mg/ml	Intratympanically into RW niche before cochleostomy
Enticott et al., 2011	Dexamethasone	0.1mg/kg	Intravenously after induction
	Methylprednisolone	125mg/ml	Intratympanically – soaked in Seprapak™ and applied to RW 30 minutes before cochleostomy
Erixon et al., 2012	Triamcinolone	40mg/ml	Not mentioned prior to RWM opening, but used topically during electrode insertion
Gstöttner et al., 2009	Triamcinolone	See note regarding dose of Volon A	Intratympanically after cochleostomy and before opening of endosteum
Gstöttner et al., 2004	Triamcinolone	See note regarding dose of Volon A	Intratympanically after cochleostomy and endosteum incised before electrode inserted
Kiefer et al., 2004			
Kiefer et al., 2005	Prednisolone	500mg	“Prior” to cochleostomy
Gstöttner et al., 2006	Triamcinolone	See note regarding dose of Volon A	Intratympanically 10 minutes after cochleostomy and before opening of endosteum, as well as to RWM
Helbig et al., 2011	Cortisone	500mg	Intravenously
	Triamcinolone	See note regarding dose of Volon A	Intratympanically 30 minutes before RWM puncture or opening of cochleostomy endosteum
James et al., 2005 (multi-centre study)	“Corticosteroid” in 9 out of 12 patients	Not documented	Intravenously “during surgery” and in one patient for 3 days prior to surgery

Author	Steroid(s) used	Dose / concentration	Route
Punte et al., 2010	Methylprednisolone	80mg	Intramuscularly prior to incision
	Triamcinolone	40mg/ml	Intratympanically after cochleostomy onto endosteum for 20 minutes and topically after endosteum opened, before electrode insertion
Rajan et al., 2011	Dexamethasone	4mg	Intravenously before intubation
	Methylprednisolone (Depo formulation)	0.6ml of 40mg/ml	Transtympanically after intubation and intratympanically during surgery before RWM opened
Santa Maria et al., 2013	Dexamethasone	12mg	Intravenously on induction
	Methylprednisolone	2. 125mg/ml (0.6 to 0.8ml)	Transtympanically at start of surgery and topically once cochleostomy opened and before electrode insertion
Skarzynski et al., 2012	“Steroid”	Not mentioned	Intravenously “during surgery”
Tamir et al., 2012	Prednisolone	1mg/kg	Intravenously at beginning of surgery
Usami et al., 2011	Dexamethasone	8mg	Intratympanically before drilling RW niche
Kuthubutheen et al., 2012	Dexamethasone	0.6mg/kg	Intravenously on induction
	Methylprednisolone (Depo formulation)	0.5ml of 40mg/ml	Transtympanically prior to skin incision and intratympanically before RWM opened

NB: In many papers, the dose of Volon A steroid is not detailed

Volon A (triamcinolone acetonide, Bristol Myers Squibb) – most commonly available at 40mg/ml.

In the study by Rajan et al., a prospective non-randomised group of patients received preoperative methylprednisolone upon induction and prior to electrode insertion (Rajan et al., 2012). The mean exposure time in adults was 80 minutes and the primary outcome measure was pure tone average (PTA) of frequencies between 125Hz and 750Hz. The steroid treatment group experienced a 19.5-dB higher mean PTA compared to the control group and this difference was statistically significant over a mean follow-up period of 20 months. In a well-designed, randomised and controlled double-blind study, the effects of a single local delivery of methylprednisolone-soaked Seprapak (125mg/ml) applied to the RW were assessed (Enticott et al., 2011). The main outcome measures were dizziness, common ground impedance and vestibular function tests, while the secondary outcome measures were audiometry and speech recognition. As in the study by Rajan et al., preoperative intravenous dexamethasone was used on induction (Rajan et al., 2012). However, unlike the study by Rajan et al., in this study, a cochleostomy was used for electrode insertion (Enticott et al. 2011). The authors reported better subjective dizziness symptoms in the steroid group as well as lower impedances in the mid-portion of the electrodes. There were no reported differences in audiometric thresholds, speech discrimination scores, or objective measures of vestibular function, such as vestibular evoked myogenic potentials or caloric response. Kiefer et al. presented results for one of the highest doses of preoperative steroids published in the CI literature to our knowledge (Kiefer et al., 2004). In this study, 500mg of intravenous prednisolone was used just prior to performing the cochleostomy. This was in addition to triamcinolone and Healon being applied to the cochleostomy site,

which was sealed using fascia through which the electrode was passed. Electrode insertion was intentionally limited to 24 mm to minimise trauma, as most patients had preserved low-frequency hearing. The group as a whole had a high degree of hearing preservation, with postoperative thresholds decreasing 5-17.5dB between 125Hz and 1000Hz.

There are a number of limitations with the three previous studies (Kiefer et al., 2004; Enticott et al., 2011; Rajan et al., 2012). In the study by Rajan et al., the groups were not randomised and a single surgeon performed all of the surgeries (Rajan et al., 2012). Hearing loss aetiology was not mentioned. Hearing preservation rates were compared at the end of the follow-up period but this was not consistent between the two groups. Furthermore, it is unclear whether there were any significant differences prior to 20 months and particularly within the first few months after implantation. There was also no mention of speech discrimination outcomes or electrophysiological parameters.

The study by Enticott et al. was well designed and the patients randomised; however, the results are difficult to compare with those of Rajan et al. (Enticott et al., 2011; Rajan et al., 2012). Different electrodes were used and insertion was via the RW. Unfortunately, “soft” surgery was not achieved in the majority of patients due to suctioning of the perilymph, incomplete electrode insertion, difficulties with the stylet, and in one case a perilymph gusher. The endosteum was also opened with a drill in the majority of cases. Therefore, it is possible the trauma from surgery may have overshadowed any hearing improvements from steroid administration. The duration of steroid application prior to

surgery was short (30 minutes). Although dexamethasone was the preferred steroid, it was not available for the entire duration of the trial.

While Keifer et al. employed the soft surgical techniques proposed by Lehnhardt et al., such as careful opening of the endosteum, their study was not primarily designed to assess the effect of steroids (Keifer et al., 2005; Lehnhardt et al., 1993). The use of much shorter insertion depths was a significant confounder and there was no control group. The study could not distinguish between high-dose systemic steroids and topical steroids, since both of these were administered.

1.7.1. Preoperative steroids – application to human cochlear implant surgery

For the transtympanic route, there is evidence from animal studies that preoperative steroids are beneficial in ameliorating the hearing loss that occurs after CI surgery. Several factors which affect this response have been characterised, including drug concentration and time of application. It remains to be seen, however, whether these factors also apply to human patients. Whilst it appears that a longer duration of exposure to steroids is beneficial, this is limited by the time constraints of surgery. In addition, the assessment of outcomes using histology is not practical. Other objective methods of assessment such as electrophysiology should be considered.

Similarly, there is evidence that administration of systemic steroids can reduce CI-related hearing loss, with histopathological evidence to support this (Connolly et al., 2011; Lee et al., 2013; Quesnel et al., 2011). Whilst the concentrations obtained in the inner ear are lower than with the transtympanic route, systemic administration avoids

many of the problems associated with middle ear dosing. The short half-life of systemic steroids may not be a disadvantage due to the prolonged genomic effects; however, its convenience needs to be balanced against the potential for side effects.

The roles of intraperitoneal and oral systemic steroids in CI surgery have yet to be studied in detail. In a guinea pig model, Liu et al. compared the perilymph concentration of dexamethasone following intraperitoneal injection of 0.5% dexamethasone (equal to 4mg/kg) with an intratympanic injection of 150uL of dexamethasone (Liu et al., 2006). Both routes achieved the same concentration in the perilymph after 30 minutes, but the intraperitoneal route resulted in a higher maximum concentration and took 30 minutes to achieve. This compared to the transtympanic route where the peak concentration was reached within 10 minutes before steadily declining. The calculated half-life of dexamethasone elimination from the perilymph was similar in both cases (2.9 hours), with the drug being undetectable after 6 hours. The time to reach maximal concentration in the study by Parnes et al. was 1 hour after intravenous injection compared to 2 hours after intraperitoneal injection (Parnes et al., 1999). The maximal concentration levels found by Parnes et al. after intravenous dosing was significantly lower than those associated with Liu et al.'s intraperitoneal dosing. From these studies, it is clear that intraperitoneal steroid administration produces a very different time-concentration curve. Perhaps the slower time to peak (2 hours versus 1 hour) allows more time for steroids to equilibrate across the blood-labyrinthine barrier, thus generating higher concentrations in the perilymph at a lower dose. This potential benefit of intraperitoneal steroids needs to be examined further.

The ideal dose for systemic preoperative steroids to have measurable effects on the inner ear in humans also needs to be studied further. An important factor to be considered is steroid potency. There is a well-known difference in potency, with dexamethasone having 6.25 to 37.5 times the anti-inflammatory activity of prednisolone. Both drugs having a very similar plasma half-life profile: 120-300 minutes for prednisolone and 150-270 minutes for dexamethasone. In order to obtain an anti-inflammatory activity similar to dexamethasone, the adult human would therefore need to ingest between 187.5mg and 1125mg of oral prednisolone. Here, potency is defined as the ability of the steroid to upregulate gene responses to glucocorticoid-induced transcription (Tanaka et al., 1994).

It is clearly impractical to perform repeated periodic sampling of perilymph following different systemic steroid doses in order to determine the optimal level. One non-invasive measure that has been suggested is to measure the increased expression of glucocorticoid receptor in peripheral blood mononuclear cells. This has previously been correlated to the expression of the receptor in the inner ear (Lu et al., 2013).

1.7.2. Hearing loss after implantation – is this influenced by preoperative steroids?

There is growing concern about the long-term stability of residual hearing preservation. Whilst the ability to preserve hearing is possible and can be optimised, long-term hearing loss is a known phenomenon. Santa Maria et al. demonstrated progressive hearing loss over 24 months following electric-acoustic stimulation surgery with a 24-mm electrode, although this did not affect speech performance (Santa Maria et al.,

2013). In their study, 12mg of intravenous dexamethasone was used on induction, followed by administration of methylprednisolone intratympanically just prior to cochleostomy. In a series of 127 full-length CI recipients, Cosetti et al. showed a hearing preservation rate of just under 30%, with no correlation between their PTA and speech discrimination (Cosetti et al., 2013). Even in patients with a 10-mm electrode, Gantz et al. showed that >30% had more than 30-dB deterioration in their PTA up to 3 years post-surgery (Gantz et al., 2009). As with the other two studies, there was no correlation between the degree of hearing preservation and word scores.

Several factors have been associated with the occurrence of delayed hearing loss. These include older age at implantation, gender and noise-induced hearing loss (Kopelovich et al., 2014). The cause of this delayed loss has been attributed to tissue response to the electrode, immune or inflammatory mediated injury, vascular injury, and even electrical stimulation. Choi and Oghalai showed that the degree of fibrosis within the scala tympani in the basal turn of the cochlea reduced the mechanical tuning at the apex of the cochlea, with greater degrees of fibrosis affecting a wider range of frequencies (Choi and Oghalai, 2005). O’Leary et al. showed that the level of fibrosis within the basal turn is correlated with hearing loss, but only at 8kHz and 16kHz, and not at 32kHz where osseous spiral lamina fracture was a factor (O’Leary et al., 2013). A number of guinea pigs in their study showed more severe hearing loss but less fibrosis, indicating the fibrotic reaction was not the only reason for hearing loss (Nadol and Eddington, 2006). Eshraghi et al. described molecular mechanisms which occur following the initial surgical trauma and which eventually lead to apoptosis (Eshraghi et al., 2013). Wright

and Roland postulated in a temporal bone study that the draining venules along the lateral wall have either minimal or no bony coverage, making them susceptible to trauma (Wright and Roland, 2013). This could then lead to stria vascularis injury, which could affect the endocochlear potential. Tanaka et al. compared guinea pigs that had been implanted and then subjected to either combined electrical and acoustic stimulation or to no stimulation (Tanaka et al., 2014). The combined stimulation group experienced greater hearing loss at a low frequency of 1kHz after 10 weeks of observation. There was also a correlation between hearing loss and strial vessel cross-sectional area and density. In addition, there was hearing loss in the absence of macroscopic evidence of hair cell loss, indicating that many effects of hearing loss are not visible by microscopy, similar to the finding of other studies (Kang et al., 2010). These observations also suggest that electrical stimulation with acoustic stimulation may contribute to hearing loss. However, the evidence that electrical stimulation from the implant causes acoustic hearing loss is variable (Coco et al., 2007).

Some of these mechanisms could be influenced by steroid administration. For example, the steroid-related increase in inner ear blood flow could compensate for strial vessel cross-sectional area or reduce strial injury (Shirwany et al., 1998). Steroids may attenuate the increased permeability of the stria vascularis which occurs after vibrational trauma (Hashimoto et al., 2006). Steroids are known to reduce the degree of fibrosis distant from the site of electrode insertion (Choi and Oghalai, 2005). Together with the known genomic effects, steroids could therefore affect long-term hearing loss related to

CI through multiple pathways. Additional studies are clearly warranted to explore this issue further.

Another long-term outcome in CI surgery which may be influenced by steroids is electrode impedance. De Ceulaer et al. utilised a triamcinolone–Healon mixture in one group and compared this to a control group (De Ceulaer et al., 2003). The steroid mixture was applied to the RW or cochleostomy site just before electrode insertion and the common ground impedance was found to be significantly lower at 2 months after surgery for the steroid-receiving group. This difference depended upon the type of electrode used. Paasche et al. injected triamcinolone into the cochlea prior to electrode insertion, resulting in lower impedances at 30 days post-CI surgery (Paasche et al., 2006). This difference was significant and persisted for up to 3 months. After 3 years, the steroid group still had lower impedances, although this difference was no longer significant (Paasche et al., 2009). In addition, the lower impedances were present in the basal portion of the electrode, closest to the site of administration.

1.8. Current gaps in the knowledge

There is currently no consensus in the literature regarding the use of steroids in hearing preservation CI and there remains considerable disagreement on many aspects of steroid use. Whilst there is some evidence of benefit from animal studies, there are few human studies on the benefits of steroids. At present, the current clinical regime employed by this author is a combination of both systemic steroids given at induction (4mg of intravenous dexamethasone) and intratympanic dexamethasone (0.5ml of 10mg/ml)

instilled in the RW niche following exposure of the RWM. A further 0.5ml of 10mg/ml of dexamethasone is instilled following opening of the RWM or cochleostomy and suctioning is avoided. Given the expanding criteria for CI and the inclusion of patients with greater degrees of residual hearing, further research into the role of preoperative steroids is warranted.

1.8.1. Duration and method of steroid administration

Based upon the above studies, there is clearly a trend towards a longer duration of steroid application prior to CI surgery. There is also a lack of evidence to support one method of steroid administration over another. The transtympanic route offers a higher concentration of steroids at the basal region of the cochlear but requires surgical exposure if round window application is utilised. The duration of surgical exposure prior to CI insertion is also limited if performed in the operating room under anaesthesia. The systemic route offers a less invasive method but potentially less absorption by the cochlea and the risk of systemic side effects. In addition, sustained high doses may be difficult to justify without any safety precedent.

1.8.2. Animal studies

A role for preoperative systemic steroids has yet to be fully established. The longest duration of preoperative steroid administration alone is 1 hour, or 6 hours if postoperative dosing is included. There is also a lack of long-term histopathological data for this indication. The current animal models for CI are arguably less applicable to human CI studies with short depths of insertion or utilisation of an electrode insertion / removal model.

1.8.3. Human studies and randomised controlled trials

Based upon the above literature review, there is clearly a lack of randomised controlled trials to assess the effect of steroids on CI. The ideal study would require subjects to be implanted with the same electrode and with the same surgical method that is consistent and atraumatic. Postoperatively, the study would require standardised postoperative testing of audiometry and speech in order to compare groups at the same time points. Ideally the study would be able to distinguish between the different routes of steroid administration and utilise the most potent steroid available for clinical use – dexamethasone.

2. Study hypothesis and aims

The aim of this thesis is to assess the role of extended preoperative steroids in CI in an animal model and in the clinical context of adult CI recipients.

2.1. Hypothesis

This study will test the following hypotheses:

- Extended preoperative steroid treatment in an animal model will reduce hearing loss associated with CI and result in favourable histopathological changes.
- Extended preoperative steroid treatment in humans will result in better hearing preservation and speech discrimination following CI.

2.2. Aims

The aims of this study are:

- To assess the role of extended preoperative systemic steroid treatment in a well-accepted and contemporary animal model of CI.
- To conduct a randomised controlled trial into the role of extended preoperative systemic steroid treatment in human CI recipients.

3. Materials and methods

3.1. Animal study – materials and methods

3.1.1. Animals

Institutional animal ethics approval was obtained from the Sunnybrook Research Institute, University of Toronto, Animal Care Committee in July 2012 (Approval number 13-499). Recognition of this ethics approval was also granted by the University of Western Australia. 24 Hartley strain albino guinea pigs [Charles River Laboratories, Canada] weighing between 614g and 1072g were used (mean 768g). There were 12 male and 12 female animals. Animals were divided into three groups, each with eight animals with an equal distribution of male and female guinea pigs.

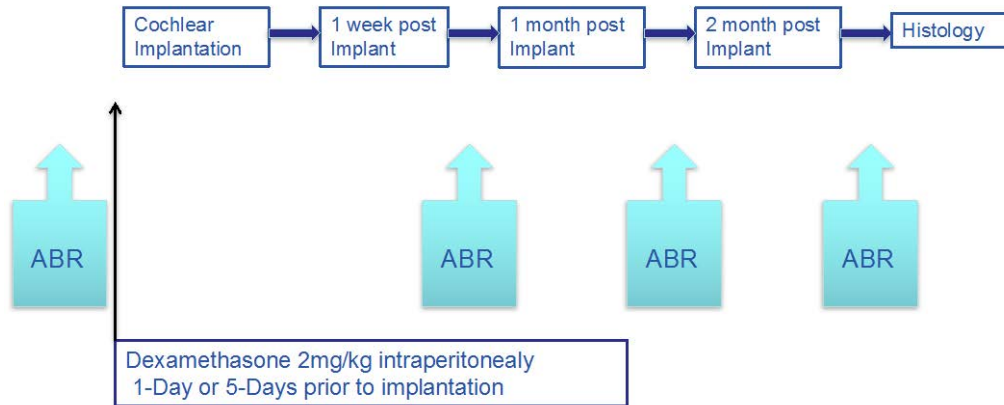
3.1.2. Testing protocol

Animals were randomly assigned to one of three groups, each containing eight animals – a control group, 1-day preoperative steroid group (“1-Day”) and a 5-day (“5-Day”) preoperative steroid group. Only the left ear was tested and subsequently implanted. ABR thresholds at 8kHz, 16kHz and 32kHz were obtained preoperatively (Figure 3.1). The control group underwent CI with no preoperative steroids. The 1-Day group received dexamethasone (2mg/kg) intraperitoneally 24 hours prior to surgery. The 5-Day group received daily intraperitoneal dexamethasone (2mg/kg) for 5 days prior to surgery. Following CI, ABR thresholds were repeated at 1 week, 1 month and 2 months post-CI. The degree of hearing loss was assessed by comparing the difference in thresholds to produce a “threshold shift”, with smaller threshold shifts indicating

reduced hearing deterioration and therefore a more favourable outcome. At the end of 2 months, the animals were sacrificed and cochleae harvested. A histopathologist blinded to the study paradigm then assessed each cochlea for all the metrics described in Histology below.

Paired student T-test were performed for within group effects and the Mann-Whitney U test and independent samples T-test were used for between group effects, with a p-value of less than 0.05 being statistically significant in all cases. Fischer's exact test was used for categorical data. All statistical analysis was performed using SPSS version 13.0 for Windows.

Figure 3.1 Diagram of animal testing protocol



3.1.3. Theory and calculation

The guinea pig cochlea typically has approximately three and a half turns, but can vary between $3\frac{1}{4}$ to $3\frac{3}{4}$ turns (Wysocki et al., 2005). The cochlear duct length is reported to range from 12 to 16mm with a mean of 14.3mm. The upper limit of hearing of the guinea

pig is 43.8kHz (Greenwood et al., 1990). Based upon the function $F=0.35(10^{(2.1/18.5)x} - 0.85)$, the three frequencies chosen for ABR testing correspond to the basal 3mm of the electrode (32kHz), the apical 2mm (16kHz) and the area 0.3mm apical to the electrode (8kHz). This is based upon an insertion 1mm from the round window. The frequencies chosen therefore assess the direct effect of electrode trauma and not effects far removed from the electrode (Figure 3.2).

Figure 3.2 Greenwood function in the guinea pig

... represents ... basal shift of CM minimum. Short vertical line represents damage to the cochlea with exposure to a 10-kHz tone (Smith and Wever, 1949). Long vertical line indicates region of swollen hair cells after long exposure to a 15-kHz tone (Neubert and Wüstenfeld, 1955). Open square: Kohlöffel's point of maximum mechanical response (*in vivo*) to 28 kHz, repeated in main graph. Main graph: Solid points represent mechanical and inner hair-cell data. Open points are based on cochlear microphonic measurements. Crosses (+) represent the CFs of primary neurons. Solid square: Kohlöffel (1972c)—28-kHz peak frequency of mechanical response at point 1.5 to 1.7 mm from base in two *in vivo* preparations. Solid triangles: Wilson and Johnstone (1975)—13 cutoff frequencies of mechanical response curves; five symbols on or touching line, with four below and four above. Solid circles: Russell and Sellick (1978, 1988 table of data points—personal communication)—CFs of 14 individual inner hair cells; six of these points are virtually on the line and, in the cluster, at about 15 mm, there are seven points, five of which are among the six on the line. Open inverted triangles: Schmiedt and Zwislocki (1978)—peak frequencies of response functions based on cochlear microphonic data. Open circles: Dallos' cochlear microphonic data as plotted by Wilson and Johnstone (1975). Crosses: Robertson and Manley (1974)—13 CFs of spiral ganglion cells versus position, points that are nearly parallel and about 0.5 mm above the line; one point is under a circle. Wilson's and Johnstone's peak frequencies also follow the

$$F = 0.35(10^{(2.1/18.5)x} - 0.85)$$

3.1.4. Auditory testing

ABR was performed using a Tucker-Davis Technologies [Florida, USA] RZ6-A-P1 Bioacoustic system for stimulus generation and data acquisition, connected to the RA4PA (4 channel preamplifier) and RA4LI (low impedance headstage). The BioSigRZ interface software was used on a PC with a P05e optical PCI express card with a fibreoptic Optibit interface. The auditory stimulus was presented through a MF1-M speaker via a 10cm tubing. ABR testing was performed in a sound attenuated and

electrically shielded animal enclosure. A ground electrode was placed on the vertex, with a positive and negative electrode placed on either ear behind the pinna.

Pure tone stimuli at 8kHz, 16kHz and 32kHz were presented. The stimulus pulse duration was 5ms with a 1ms rise and fall, presented at a rate of 21 pulses/second with a period of 47.619ms and a Blackman envelope. The recording envelope was 10ms with a 20x gain of the signal. A low pass filter of 3kHz and a high pass filter of 300Hz were used in addition to a notch filter of 60Hz. A minimum of 500 averages was performed in order to obtain a reproducible ABR waveform. Threshold testing was performed in 2 down, 1 up 5-dB step paradigm and the ABR threshold was defined as the stimulus level below which there was disappearance of the wave V. For ABR testing, animals were anaesthetised using a combination of nitrous oxide gaseous induction and combination intraperitoneal ketamine [Vetalar 100mg/ml, Bioniche Canada] and xylazine [Rompun 100mg/ml, Bayer HealthCare, Canada] for maintenance.

3.1.5. Cochlear implant surgery

A custom-made CI silicone electrode [Med-El GMBH, Austria] was utilised. The electrode was tapered and sequentially marked with the diameter being 0.3mm at the tip and 0.5mm at 5mm. The electrode was sterilised in ethylene oxide and double wrapped. CI was performed under nitrous oxide anaesthesia only and using a sterile technique. The postauricular area of the left ear was shaved and prepped with non-alcoholic surgical strength Betadine [Purdue Products LP]. 1% xylocaine with 1:100,000 epinephrine was used to infiltrate the postauricular skin area. A vertical incision was made and the skin and postauricular muscles were divided to expose the surface of the

bullae. A 3mm cutting burr attached to a Bein-Air otologic burr [Switzerland] was used to open the bulla to expose the tympanic annulus, basal turn of cochlea, and round window. A 0.6mm diamond burr was used to create a cochleostomy, 1mm from the edge of the round window bony lip. The electrode was then inserted until the 5mm mark. A small muscle plug was used to cover the cochleostomy site. The wound was then closed in two layers using 4-0 Polysorb [Syneture] braided absorbable suture. No intravenous or systemic antibiotics were given apart from daily wound application of topical Polysporin ointment [(Johnson and Johnson Inc.) for 5 days.

3.1.6. Cochlear implant electrode

The following diagram (Figure 3.3) is a picture of the custom-made guinea pig CI electrode.

Figure 3.3 Guinea pig CI electrode [Source: MedEL Austria]

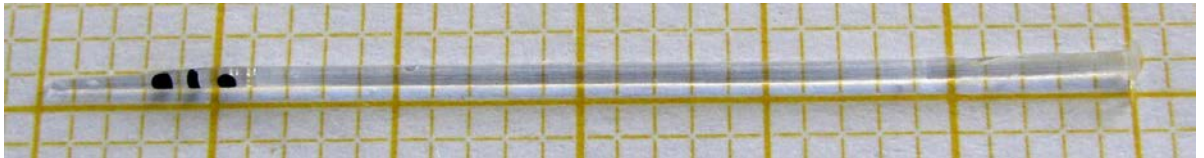
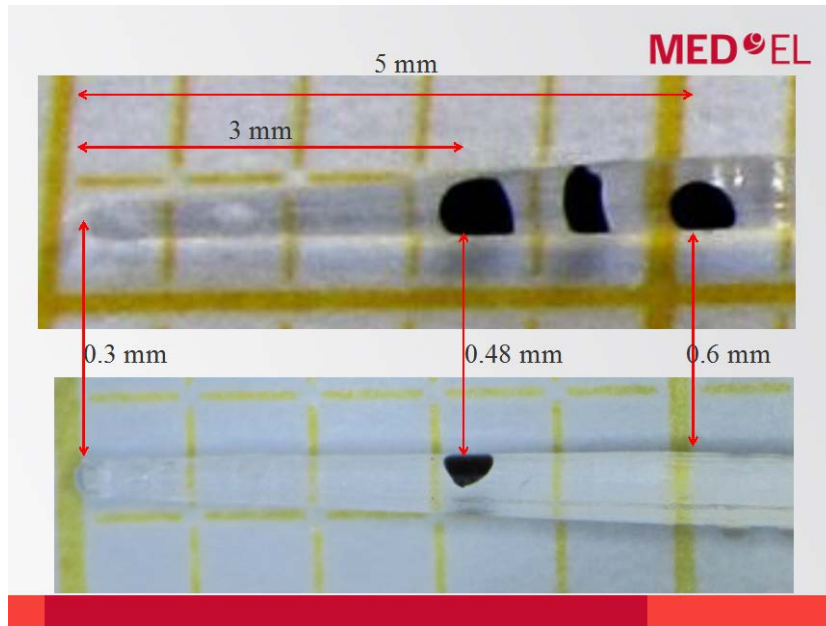


Figure 3.4 shows a close-up picture of the same electrode at its proximal end. Note the markers at 3 and 5mm as well as the corresponding diameters at the tip, and at 3 and 5mm.

Figure 3.4 Guinea pig CI electrode, close up with measurements



3.1.7. Histology

For histological assessment of the cochlea, animals were euthanised with sodium thiopental intracardiac injection followed by harvesting of the cochlea. The electrode and stapes footplate were removed and the cochlea placed immediately in 4% paraformaldehyde. The cochleae were then decalcified in 4% EDTA, paraffin embedded, sectioned and stained with haematoxylin and eosin. For each specimen, four sequential sections were taken, with each section being 5 μ m in thickness. Sections were cut parallel to the cochlea modiolus. If any specimens were not readable, further sections were taken. For each section, a single profile of the organ of Corti was examined per cochlear turn (apical, mid and basal). The mean results of the four sections for each animal were then recorded. The following measures were taken for each section:

- Area of implant related fibrosis in the basal turn (mm²).
- SGN density in cells per mm² in the basal, mid and apical turns.

- IHC counts in the basal, mid and apical turns.
- OHC counts in the basal, mid and apical turns.

The area of implant fibrosis was defined as the visible area of fibroblast reaction between the electrode tract and the osseous spiral lamina and all areas were measured using a micrometre. For each section, all three organ of Corti profiles in the apical, mid and basal turns were examined for the presence of foreign body giant cells, osteoneogenesis, and fractures of the osseous spiral lamina. The basal turn in each section was assessed for the presence or absence of a visible electrode tract.

3.2. Human study – materials and methods

3.2.1. Subject recruitment

The study methodology utilised the CONSORT principles of randomised controlled trial design. Institutional ethics approval was obtained from the Sunnybrook Research Ethics Board in July 2012 and the study was conducted at a Sunnybrook Health Sciences Centre, University of Toronto, an adult tertiary implant centre in Ontario, Canada (Approval number 184-2012). Post-lingual deaf adult patients were recruited between December 2012 and January 2014. Subjects recruited were aged between 18 and 85 years of age, considered medically suitable for CI surgery and had no central pathology. Audiometric inclusion criteria were pure tone thresholds of better than or equal to 80dB at 125Hz and 250Hz and better than or equal to 90dB at 500Hz and 1000Hz. Speech discrimination inclusion criteria was determined by the HINT (Hearing in Noise Test) score of less than 60% in quiet in the best-aided condition at 60dB SPL. Duration of

deafness was not considered in the inclusion criteria. Audiometric inclusion criteria were chosen to reflect the typical patients seen in our CI clinic and were not candidates for electric acoustic stimulation.

Subjects were randomised to one of three groups using a random list generator [www.random.org]. The control group received standard CI surgery (“control” group). The second group received a course of oral prednisolone at a dose of 1mg/kg/day up to a maximum dose of 60mg per day for 6 days prior to and including the day of surgery (“oral” group). The third group received a single 0.5ml transtympanic dose of dexamethasone (10mg/ml) 24 hours prior to surgery (“transtympanic” group).

Subjects were notified of their allocated group by the primary author and two co-authors who were both un-blinded to the treatment condition. The surgeons performing the surgery were blinded to the treatment group preoperatively but not postoperatively. Audiologists performing audiometric testing and CI mapping were blinded preoperatively by seeing patients prior to randomisation and blinded postoperatively. Subjects were asked not to reveal their treatment group to the treating audiologist and records of their intervention were kept in a separate location. Patients could not be blinded to the intervention because there was no placebo utilised and interventions involved either an injection or taking medication. If a patient was randomised to receive oral prednisolone but could not do so due to a medical contraindication such as diabetes, they were then randomised to either the control or transtympanic group. Patients who

were recruited but subsequently dropped out or who were lost to follow up were documented.

The sample size was calculated based upon a population size of 150 patients (our annual CI candidate intake) and being able to detect at least a 15% change in audiometric thresholds and speech discrimination scores with a 95% confidence interval. The recommended sample size was 34 patients, making this study adequately powered [www.raosoft.com].

3.2.2. Treatment details

Oral prednisolone was chosen because it was our systemic steroid of choice for the treatment of sudden sensorineural hearing loss. Patients were prescribed oral prednisolone using a pharmaceutical script and were instructed to take the first dose 6 days prior to surgery. Patients were counselled about the potential side effects and compliance was assessed verbally just prior to surgery. Dexamethasone was chosen as the steroid for the transtympanic group because it was our steroid of choice for the treatment of sudden sensorineural hearing loss that is refractive to systemic steroid treatment. The transtympanic injection was performed 24 hours prior to surgery. The posterior-inferior quadrant of the tympanic membrane was anaesthetised with topical phenol. A 3.5-inch (90mm length), 25-gauge (0.50mm diameter) spinal needle attached to a 1ml syringe was then used to infiltrate 0.5ml of 10mg/ml concentration dexamethasone sodium phosphate [Omega Healthcare, Montreal, Canada]. Infiltration of steroid into the middle ear space was confirmed by visualising a rising fluid level

behind the tympanic membrane. Patients were then asked to tilt their head towards the side of injection and lie supine for 15 minutes.

Surgery was performed by the attending surgeon in all cases with assistance from the resident and fellow. In all cases, a standard postauricular incision, creation of a cortical mastoidectomy and a facial recess approach. In all groups, the anaesthetist administered 10mg of dexamethasone and 2g of cephazolin intravenously on induction. In all groups, topical dexamethasone (10mg/ml) was also applied just prior to opening of the RWM in all patients as part of our standard surgical procedure. All surgeons were blinded to the treatment group.

Once the RWM was opened, suctioning of the perilymph was avoided. In all cases, the round window approach was used to insert the electrode until full insertion was achieved or resistance on further insertion was felt. Soft tissue was used to obliterate the RW niche to prevent postoperative perilymph leakage. All patients received a Med-EL [Innsbruck, Austria] CONCERTO CI with a Flex-Tip electrode, with each electrode having 12 contacts numbered sequentially from 1 (apical) to 12 (basal).

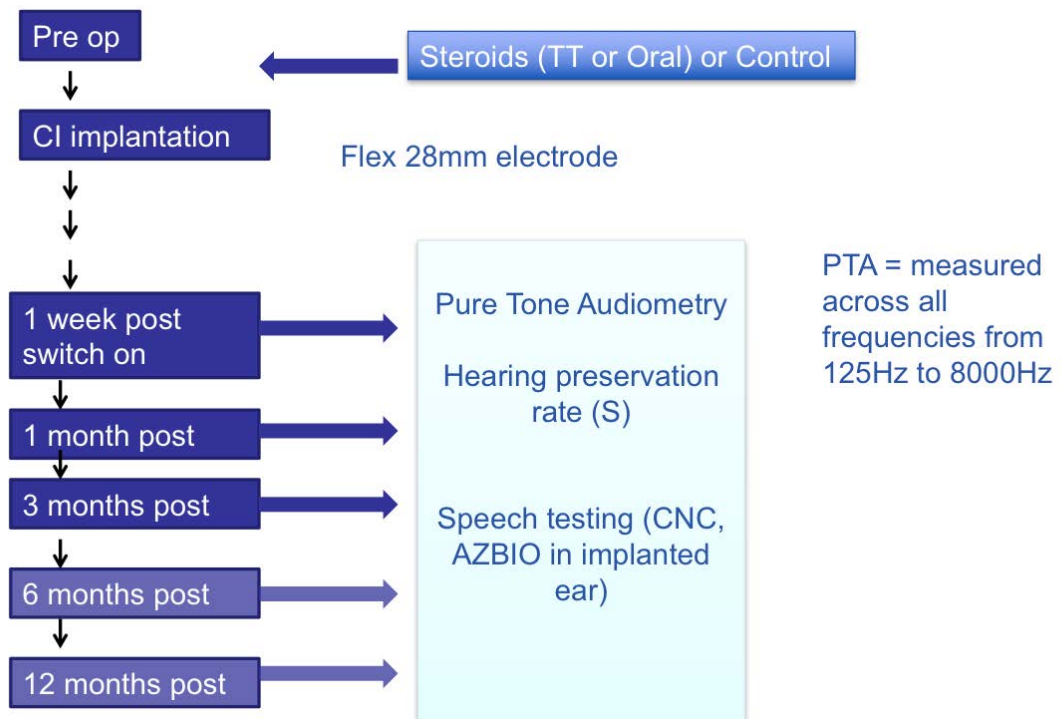
The implanted electrodes were either 24mm, 28mm or 31mm in length. The electrode length was chosen after the patients were randomised into the study group and was based upon the decision of a multidisciplinary implant group meeting who were blinded to the study. This group comprised of both surgeons and audiologists who were blinded to the treatment group preoperatively, hence the electrode length chosen was independent of

the treatment received. Only patients who had been implanted with a 28mm electrode were included in the final analysis.

3.2.3. Postoperative testing protocol

Pure tone audiometry was measured from 125Hz to 8000Hz. Word discrimination was tested in the best-aided scenario using the Consonant Nucleus Consonant (CNC) open set monosyllabic word test in quiet presented at 60dB SPL (Petersen and Lehisté, 1962). Sentence testing was performed using the AZBio sentence test in quiet at 60dB SPL in the best-aided situation and CI-only situation (Spahr and Dorman, 2004). These tests were repeated at 1 week, 1 month, 3 months, 6 months and 12 months post-implant activation. All implants were activated at 1 month post-surgery as per our routine practice (Figure 3.5).

Figure 3.5 Diagram of human testing protocol



3.2.4. Data analysis

Audiological thresholds were assessed two-fold. The first involved assessing the PTA across the inclusion criteria low frequencies (125Hz, 250Hz, 500Hz and 1000Hz), or low frequency PTA (LF-PTA). The second involved assessing the PTA across all frequencies from 125Hz to 8000Hz or all frequency PTA (AF-PTA). When no measurable hearing was recordable, the maximum output of the audiometer was assigned rather than excluding this frequency. This avoided artificially lowering the observed mean thresholds. Hearing preservation was calculated using the formula proposed by a recent consensus group paper (Skarzynski et al., 2013). The PTA was first calculated in a similar manner across frequencies from 125Hz to 8000Hz and changes to the PTA normalised to the patient's initial hearing and the audiometer frequency specific maximum output was then used to calculate the S value (percentage of hearing preservation). Therefore, the higher the S value the greater the degree of hearing preservation, as represented by the following formula:

$$S = \left[1 - \left(\frac{PTA_{\text{post}} - PTA_{\text{pre}}}{PTA_{\text{max}} - PTA_{\text{pre}}} \right) * 100 \right] [\%]$$

Statistical analysis was performed using SPSS version 24.0. Repeated measures ANOVA (analysis of variance), MANOVA, independent samples t-test (Mann-Whitney U) and Fisher's exact test were used to evaluate differences between groups.

4. Results

4.1. Animal study

4.1.1. Auditory threshold results

The control group results are presented in Table 4.1. At 1-week post-implantation, the threshold shifts at 8kHz, 16kHz and 32kHz were all significantly different to the preoperative state ($p < 0.05$). The largest threshold shift was at 32kHz. At 1-month post-surgery, the corresponding threshold shifts were significantly different compared to the preoperative condition at 8kHz and 32kHz, with the largest threshold shift observed at 8kHz (apical). At 2 months, the threshold shift was significantly different compared to the preoperative condition only at 16kHz. Although there was recovery in hearing thresholds following the acute hearing loss, hearing at 2 months did not approach the preoperative hearing level.

Table 4.1 Threshold shifts (from preoperative hearing level) across all three groups

	Mean dB threshold shift at 1 week			Mean dB threshold shift at 1 month*			Mean dB threshold shift at 2 months*		
	8kHz	16kHz	32kHz	8kHz	16kHz	32kHz	8kHz	16kHz	32kHz
Control	27.5* (6.0)	26.9* (17.9)	33.1* (11.9)	19.4* (14)	8.75 (18.1)	11.9* (13.1)	21.25 (19.2)	15* (21.7)	6.25 (6.94)
1-Day	20.6 (7.3)	13.1 (10.7)	13.8** (14.6)	15 (11.3)	5.6 (7.3)	5 (9.3)	17.5 (7.1)	8.75 (13.6)	6.9 (13.9)
5-Day	22.5 (15.6)	14.4 (14.5)	9.4** (7.3)	18.1 (13.3)	13.8 (12.2)	7.5 (12.2)	16.9 (16.2)	7.5 (12.2)	5.6 (7.3)

The numbers in brackets indicate the SD

* Paired t-test statistically significant compared to preoperative ($p < 0.05$)

** Mann-Whitney U test statistically significant compared to control group ($p < 0.05$)

The 1-Day steroid group results are presented in Table 4.1. At 1-week post-surgery, the threshold shift was significantly smaller compared to the control group at 32kHz. At both 1 month and 2 months, threshold shifts were smaller than the control group, but these differences were not significantly different.

The 5-Day steroid group results are presented in Table 4.1. At 1-week post-surgery, the threshold shifts compared to the control group were smaller across all frequencies, and significantly smaller at 32kHz. Compared to the 1-Day steroid group, the 5-Day group had a smaller threshold shift at 32kHz, although this was not significant. At 1 month, the threshold shifts were smaller than the control group (apart from 16kHz), but these were not significantly different. At 2 months, the threshold shifts were smaller than the control group but again they were not significantly different.

4.1.2. Histology results

The histopathological results for all three groups after 2 months are shown in Table 4.2. Histopathological data was assessable for 20 of the 24 animals. The remaining four animals (two each from the control group, and the 1-Day steroid group) had unreadable histopathological slides due to damage during cochlea harvesting. In almost all cases, the electrode tract was visible within the basal turn of the cochlea, indicating correct positioning of the electrode (Figure 4.1). Where the eccentricity of the fibrotic response could be identified, this was always centred in the medial half of the scala tympani and towards the modiolus.

Figure 4.1 Typical section showing electrode tract within the scala tympani and surrounding fibrosis (haematoxylin and eosin stain)

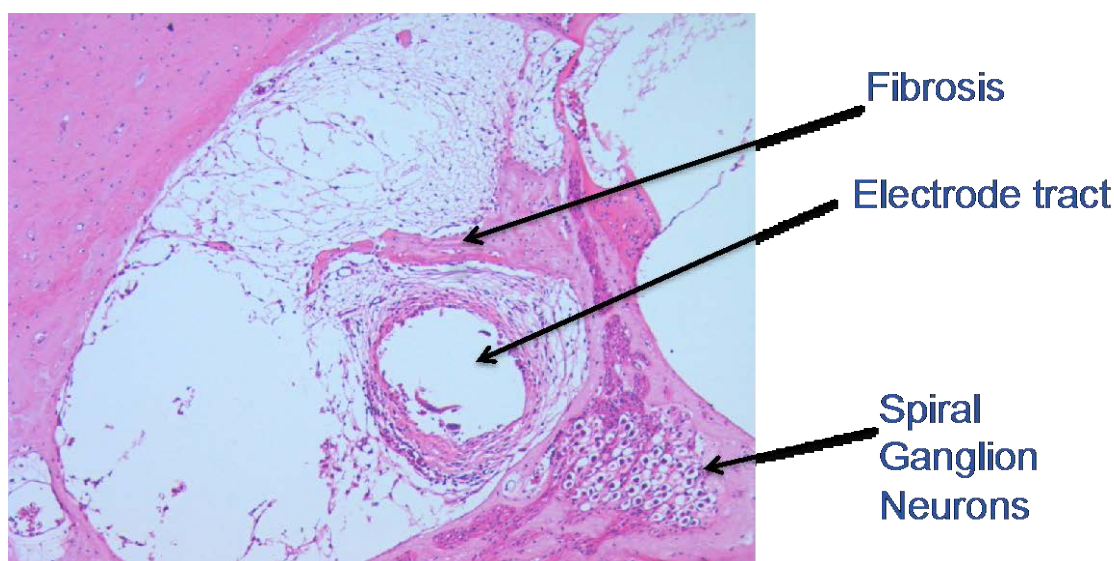


Table 4.2 Histopathological results for all three animal steroid groups

	Foreign body giant cells presence	Osteoneogenesis presence	Osseous spiral lamina fracture presence	Electrode tract identifiable
Control	50% (3 out of 6)	33.3% (2 out of 6)	0% (nil)	83.3% (5 out of 6)
1-Day	66.7% (4 out of 6)	50% (3 out of 6)	0% (nil)	66.7% (4 out of 6)
5-Day	63% (5 out of 8)	25% (2 out of 8)	0% (nil)	75% (6 out of 8)

The presence of foreign body giant cells, osteoneogenesis and osseous spiral lamina fracture were all assessed. Using the Fischer exact test, no statistically significant differences were apparent amongst the three groups for these parameters ($p>0.05$).

Table 4.3 shows the mean SGN densities at the basal, mid and apical turns for each group, as well as the surface area of electrode-related fibrosis. The mean area of electrode-related fibrosis was 0.43mm^2 in the control group, 0.3mm^2 in the 1-Day group, and 0.35mm^2 in the 5-Day group. Although the 1-Day group had the smallest area of

electrode-related fibrosis, this was not significantly different when compared to the control group ($p>0.05$).

The mean basal turn SGN density was 707 cells/mm² in the control group, 663 cells/mm² in the 1-Day group, and 1096 cells/mm² in the 5-Day group. Between-group ANOVA analysis showed the 5-Day group had significantly higher SGN densities than both the control group and the 1-Day group ($p<0.01$), with no difference between the 1-Day and control groups. The mean mid turn SGN density was 1008 cells/mm² in the control group, 833 cells/mm² in the 1-Day group and 995 cells/mm² in the 5-Day group. The lower density in the 1-Day group compared to control only approached significance ($p<0.08$). The 5-Day group tended to have higher densities than the control group when values were averaged across all cochlear turns, but there was no statistically significant difference between groups.

Table 4.3 Electrode related fibrosis and SGN densities for all three groups

	Mean electrode-related fibrosis (mm ²)	SGN density at basal turn (cells/mm ²)	SGN density at mid turn (cells/mm ²)	SGN density at apical turn (cells/mm ²)
Control	0.43 (0.26)	707 (136)	1008 (168)	945 (168)
1-Day	0.3 (0.17)	663 (153)	833* (202)	867 (208)
5-Day	0.35 (0.31)	1096** (285)	995 (240)	1066 (156)

The numbers in brackets indicate the SD

* Independent samples T test, $p=0.08$ compared to control

**Mann-Whitney U test, $p<0.05$ compared to control and 1-Day

Across all animals, bivariate analysis showed a negative correlation between basal SGN densities and the threshold shifts at all time points and across all frequencies i.e. larger threshold shifts were associated with lower SGN densities (Table 4.4). However, this was only significant for the 2-month postoperative threshold shift at 8kHz ($R=-0.604$, $p<0.05$) and 16kHz ($R=0.493$, $p<0.05$), with a larger R-value in the 8kHz region (Figures 4.2 and 4.3). There was no statistically significant correlation between the area of fibrosis and threshold shifts across all time points and across all animals.

Figure 4.2 8kHz threshold shift at 2 months versus basal SGN density at 2 months (cells/mm²)

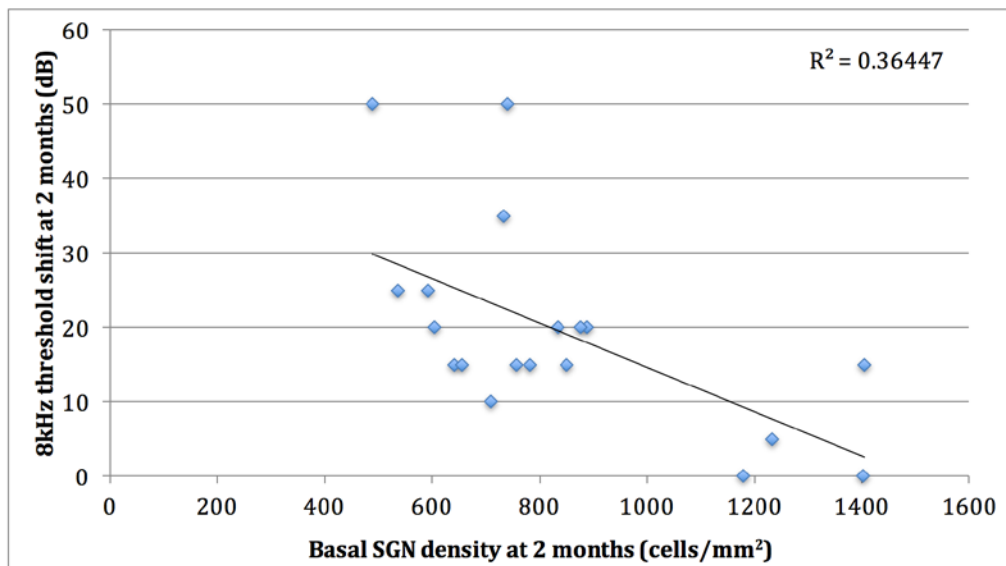


Figure 4.3 16kHz threshold shift at 2 months versus basal SGN density at 2 months (cells/mm²)

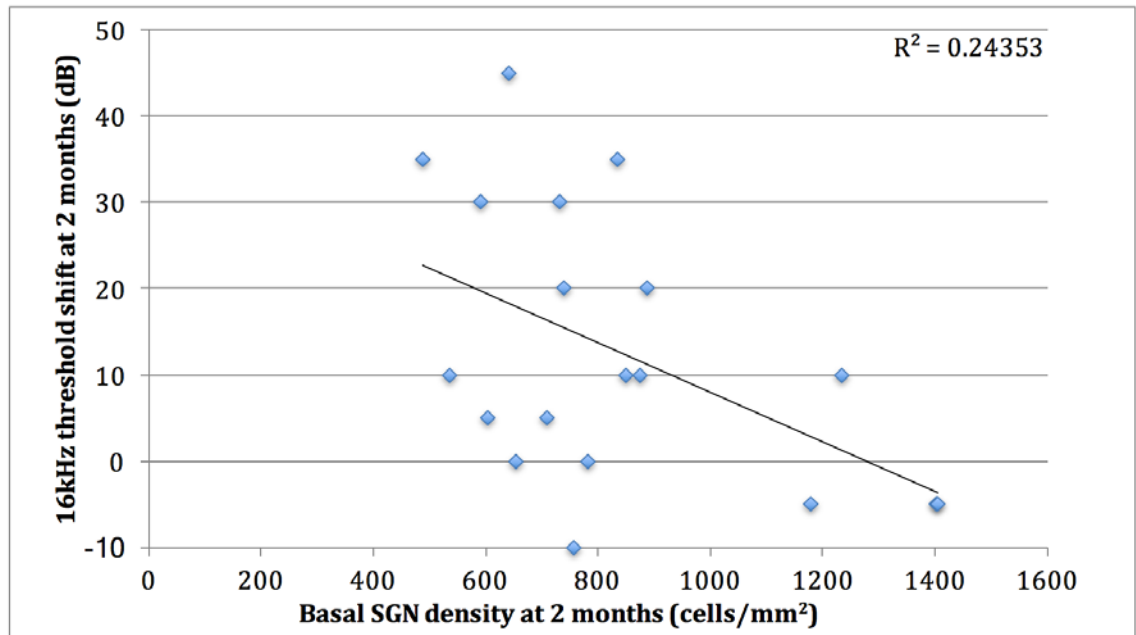


Table 4.4 Pearson correlation co-efficient values between SGN density (cells/mm²) and threshold shifts at 1 week, 1 month and 2 months (in dB compared with control)

SGN density	Threshold shift at 1 week*			Threshold shift at 1 month*			Threshold shift at 2 months*		
	8kHz	16kHz	32kHz	8kHz	16kHz	32kHz	8kHz	16kHz	32kHz
Basal spiral	-0.46	-0.291	-0.181	-0.365	-0.212	0.20	-0.604	-0.493	-0.31
Mid turn	-0.153	0.129	0.514	-0.434	-0.208	-0.150	-0.443	-0.250	-0.301
Apical spiral	0.258	0.301	0.159	-0.136	-0.335	-0.056	0.100	0.206	-0.093

Values in bold indicate statistical significance according to the Mann-Whitney U test ($p < 0.05$)

The mean IHC and OHC counts for each group are shown in Table 4.5. Unfortunately, due to an artefact of tissue processing, the apical turn hair cells were not assessable in the 5-Day group. There appears to be a much wider variability in results for the 1-Day group compared to the other two groups, as indicated by the relatively larger standard

deviations (SDs). The 5-Day group had significantly higher IHC counts in the mid turn of the cochlea compared to the control group ($p=0.01$), but with no other differences to the control group. Surprisingly, however, the 1-Day group had significantly lower IHC counts compared to the control group in the basal turn and lower OHC counts in the mid turn and basal turns. All animals survived to 2 months with no incidence of infection (as manifested by fever, wound cellulitis or meningitis).

Table 4.5 Mean IHC and OHC counts for each of the three groups at the basal, mid and apical turns of the cochlear

Group	Mean IHC count			Mean OHC count		
	Basal turn	Mid turn	Apical turn	Basal turn	Mid turn	Apical turn
Control	1 (0)	0.83 (0.38)	0.58 (0.50)	2.75 (0.44)	2.8 (0.48)	2.1 (0.61)
1-Day	0.55 (0.49)	0.65 (0.49)	0.65 (0.49)	2.3 (0.95)	2.2 (1.2)	1.85 (1.09)
5-Day	0.94 (0.25)	1 (0)	N/A	2.7 (0.64)	2.97 (0.18)	N/A
Control vs. 1-Day p-value	0.001*	0.17	0.66	0.04*	0.02*	0.298
Control vs. 5-Day p-value	0.22	0.01*	N/A	0.68	0.15	N/A

The numbers in brackets indicate the SD

Independent sample T-test was used to compare between groups

Bold figures indicate a statistically significant difference ($p<0.05$)

N/A indicates that apical most hair cells could not be assessed in the 5-Day group of animals

4.1.3. Discussion

Preoperative drug administration is an attractive option in hearing preservation CI because it aims to protect the inner ear prior to opening the inner ear, as opposed to after insertion of the electrode. The difficulty lies in how to best deliver the steroid to where it is required in the least traumatic manner, with the highest concentration, for the longest

duration, and with the least side effects. To our knowledge, the intraperitoneal route of systemic preoperative steroid administration has not been studied in animal models of CI. Liu et al. compared the pharmacokinetics of a single dose of 0.5% intraperitoneal dexamethasone (equivalent to 4mg/kg) to a single application of 150uL intratympanic dexamethasone (Liu et al., 2006). The authors found the intraperitoneal route reached its peak concentration in the perilymph after 2 hours compared to 10 minutes for the intratympanic route. This was despite both having a similar perilymph elimination half-life of 2.9 hours and both being undetectable after 6 hours in the perilymph. However, the intraperitoneal route reached a significantly higher mean maximal concentration (0.927mg/L) compared to the intratympanic route (0.237mg/L). In a study by Parnes et al. where a higher single intravenous dexamethasone dose of 8mg/kg was used, the peak concentration in the perilymph was reached 1 hour after administration (Parnes et al., 1999). However, this was achieved at a lower concentration of 0.220mg/L compared to the intraperitoneal route by Liu et al. These studies suggest that intraperitoneal dexamethasone is able to cross the blood labyrinthine barrier. In comparison to the intravenous route, the intraperitoneal route may be able to achieve a higher concentration of drug in the perilymph at a lower dose and over a longer period of time. Whilst the longer time to peak concentration may be due to slower drug absorption from the intraperitoneal space into the intravascular space, the reason for the higher perilymph concentration is uncertain. Perhaps there is a longer time available for the drug to equilibrate across the blood labyrinthine barrier, or there may be changes to the

permeability of this barrier induced by the intraperitoneal injection route. Further studies are required to investigate these different possibilities.

The results of our study suggest that systemic steroids when given up to 24 hours prior to surgery seem to offer greater hearing protection effects at the basal turn of the cochlea. This was demonstrated by the significantly smaller threshold shifts in both steroid groups at 32kHz. There are a number of possible reasons for this frequency selectivity. In the murine model, high dose systemic steroids have been shown to induce a gradient of uptake within the organ of Corti from base to apex, with greater uptake observed at the basal turn of the cochlea (Grewal et al., 2013). Two significantly higher doses of steroid were used (10mg/kg and 100mg/kg) in the study by Grewal et al. By 6 hours, a decreasing basal to apical gradient was noted, which was absent after 12 hours. Another possible explanation for the differential uptake from systemic administration is regional differences in the blood supply in the cochlea. Corrosion casting of the cochlea in the pig and chinchilla model have found regional variations in the stria vascularis morphology. These differences included variances in the density of the capillary network, branch patterns, degree of tortuosity, and vessel diameter (Carraro et al., 2013). Glucocorticoids have also been shown to increase blood flow to the cochlea (Shirwany et al., 1998), adding weight to this hypothesis.

Although the 5-Day group had significantly higher basal SGN densities, the correlation between basal SGN densities and threshold shifts at 8kHz and 16kHz but not at 32kHz suggests the basal SGN population cannot be assumed to be the direct site of action of

dexamethasone. Other potential mechanisms need to be considered, including the effect of dexamethasone on systemic immunity. The role of systemic immunity in implant-related hearing loss was demonstrated by Souter et al. who showed that guinea pigs primed with a sterile antigen had hearing loss over a broader range of frequencies and developed a greater degree of fibrosis compared to controls (Souter et al., 2012).

Nonetheless, the high frequency hearing protective effects seen in the model used here appear to be short-lived, with the effects present at 1 week but no longer significant at 1 month. This suggests that at the studied dose, systemic steroids may only be protective against the electrode insertion trauma, if trauma is defined by immediate deterioration in audiological thresholds.

The dexamethasone dosage of 2mg/kg used in the 1-Day group has previously been used in two published studies with no deleterious effects on auditory thresholds (Connolly et al., 2011; Lee et al., 2013). However, in Lee et al.'s paper the systemically treated group had lower OHC counts. This finding is similar to our observation in the 1-Day group. Our results showing deleterious effects of steroids on both IHC and OHC at 2 months in the 1-Day group is somewhat surprising. We found the 1-Day group had lower OHC compared to the control group at the basal and mid turns, and lower IHC at the basal turn. In contrast, the 5-Day group had higher IHC at the mid turn. The lower hair cell counts observed in the 1-Day group may be due to artefact, as suggested by the large SD. Nonetheless, this apparently deleterious effect of steroids on hair cells should not be discounted and requires further assessment.

The daily dose of 2mg/kg for 5 days was chosen in an attempt to obtain a condition of prolonged exposure to dexamethasone prior to implantation. The use of prolonged doses of intraperitoneal dexamethasone in guinea pigs has been previously published. A dose of 10mg/kg of intraperitoneal dexamethasone was used for 7 consecutive days to assess the effect on glucocorticoid receptor mRNA expression and protein levels (Lu et al., 2013). In the study by Lu et al., glucocorticoid receptor levels were not suppressed by dexamethasone and in fact the converse was observed. In another study by Yu et al., an intraperitoneal dexamethasone dose of 5mg/kg was used for 7 consecutive days in guinea pigs to assess the effect on aquaporin-1 expression in otitis media with effusion (Yu et al., 2013). Wang et al. showed that 1mg/kg of intraperitoneal dexamethasone for 5 consecutive days in guinea pigs was protective against noise induced hearing loss (Wang et al., 2011a, b).

Dose equivalence studies suggest the dose of 2mg/kg used in our study is equivalent to a human dose of 0.434mg/kg, or approximately 30mg in a 70kg adult (Center for Drug Evaluation and Research, 2005; Connolly et al., 2011; Reagan-Shaw et al., 2008). This dose is higher than the standard dexamethasone dose of 10mg given at our institution by anaesthetists for nausea, but is greater than the dose given for severe head and neck inflammation (8mg TDS or 24mg daily). Significantly higher doses than this have been used in a study on hearing preservation surgery where a preoperative dose of 500mg prednisolone was given intravenously in 14 patients with no reported side effects (Kiefer et al., 2004). This dose of prednisolone is equivalent in potency to 250mg of

dexamethasone (Tanaka et al., 1994). Caution should still be exercised, however, when extrapolating the doses used in our study for use in humans.

In the present study we found that both steroid treatment groups had smaller threshold shifts at 32kHz. However, the two groups differed in their hair cell counts and SGN densities. It is possible that the duration of steroid exposure may have altered the response of the inner ear to electrode-related trauma. Another potential explanation is that a single dose of dexamethasone 24 hours prior to surgery was sufficiently protective of hearing in the short-term, but failed to protect against hair cells losses from occurring in the longer term. The higher basal turn SGN density and higher mid turn IHC counts in the 5-Day steroid treated groups suggest that a longer duration of preoperative systemic steroids may be preferable to a single preoperative dose.

Our results on the effects of steroids on SGN density are similar to a study where these effects were seen at 3 months post-CI but not at 1 month (Maini et al., 2009), suggesting it takes a longer to observe effects on SGN populations compared to effects on auditory thresholds. The importance of preserving SGN function is further supported by the correlation between SGN densities and threshold shifts. However, the positive correlation between mid turn SGN density and the 32kHz threshold shift at 1 week is probably misleading because of the differences in timing of each measurement. The SGN protective effect of steroids is promising because these cells are the eventual targets for electrical stimulation by the CI electrode. It should be noted that SGN density only assesses cellular microscopic appearance and not function, which may be altered by

steroids. It is well known that glucocorticoid receptors are present in the SGN and throughout the cochlea (Rarey et al., 1996). One way to assess functional status of the SGN is to measure the electrically evoked compound action potential (ECAP), which can be performed by most current CIs.

This study was performed in normal hearing animals and hence the extrapolation of findings to pathologies such as noise-induced ototoxicity or age-related hearing loss should be done with caution. For example, age-related hearing loss (at least in humans) usually presents with significant high frequency hearing loss, thus rendering less relevant any potential benefit from systemic steroids on high frequency residual hearing. High frequency hearing loss, or ski-slope hearing loss amenable to combined electroacoustic stimulation (EAS) CI may also mask any audiometric threshold improvements from systemic steroids. However, despite any measurable audiological benefits, there may be steroid-related changes in electrophysiology such as in the ECAP response or in electrocochleography (ECOG).

This study is also relevant to the current trend towards “soft” surgery. A slim silicone electrode was used in this work which has a high degree of flexibility that facilitated atraumatic electrode insertion. The smallest possible cochleostomy was used and no suctioning of perilymph was performed. A deeper insertion was also performed compared to previously published studies (James et al., 2008) and the electrode was left in situ rather than removed after insertion (Eshraghi et al., 2007; van der Water et al., 2010). The atraumatic insertion of this technique was reflected by the partial reversal of

threshold shifts in the control group. Our model is also similar to that recently described (Giordano et al., 2014).

The tendency toward a beneficial effect of steroids on electrode-related fibrosis observed in our study is somewhat promising, considering the lack of statistical significance across groups may be due to the small sample size. The lack of correlation between degree of fibrosis and hearing in this study suggests that fibrosis is not the only determinant in hearing preservation. This may be due to the location of the fibrosis within the scala tympani not interfering with the organ of Corti. However, in the context of human CI, the degree of fibrosis may materially affect electrical impedance which may affect the amount of current required to stimulate the SGN. An important implication of this is the impact on improving speech processor battery life.

No infective complications were noted during this study, suggesting that steroid use does not result in a higher incidence of infection. Systemically administered steroids are not without side effects; however, including elevations in blood glucose levels, gastrointestinal effects, increased appetite, euphoria, suppression of the hypothalamic pituitary axis (for longer dose durations) and femoral head necrosis (Weinstein et al., 2012). These potential side effects may be limited by short duration of use and should always be a consideration in human applications.

Future animal studies should include testing higher doses of steroids, electrophysiological measurements (such as impedance and ECAPs) and other objective measures of hair cell function (such as otoacoustic emissions and cochlear

microphonics). The results of this study support the use of preoperative steroids to complement other methods of steroid delivery under development, such as steroid-eluting electrodes. The concept of “complete steroid coverage” could then be developed and evaluated to determine if preoperative steroids are synergistic with other methods of drug delivery.

4.1.4. Conclusion

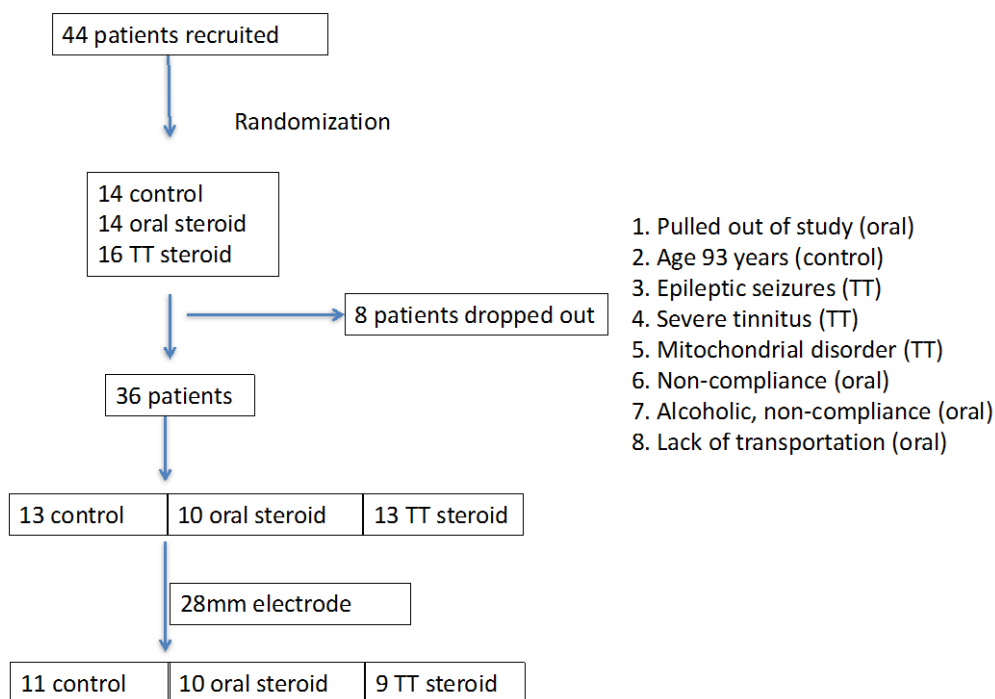
In our animal model, preoperative intraperitoneal dexamethasone showed significant reduction in threshold shift on ABR at high frequency (32kHz). There was also increased SGN density limited to the basal turn of the cochlea.

4.2. Human study

4.2.1. Patient flow

The flow of patients from recruitment to 12-months is shown in Figure 4.4. A total of 44 patients were recruited and randomised into three groups. Eight patients were excluded after receiving CI surgery. Of these, one patient voluntarily withdrew, two were non-compliant with follow up and one patient fell outside the age criteria shortly after recruitment. In addition, one patient had seizures during the testing period, one was found to have a mitochondrial disorder, one patient had disabling tinnitus postoperatively, and one patient had no reliable transport.

Figure 4.4 Patient flow diagram showing the recruitment pathway of patients and the final cohort included in the analysis



The mean age of the excluded group was 61 years (SD 20.3), with five females and three males. There were no significant differences between the age or sex distribution of this group and the main cohort. The mean duration of deafness of this group was 22.6 years (SD 14.7), which also did not differ from the main cohort. One patient was allocated to the control group, four to the oral group and three to the transtympanic group. The aetiologies of hearing loss were similar to the main cohort. Equal numbers of left and right ears were implanted in the excluded group (Table 4.6).

After the initial exclusion of eight patients described above, the cohort total was 36 patients comprised of 30 patients implanted with a 28mm electrode, three patients with a 31mm electrode and three patients with a 24mm electrode. Only patients with the 28mm

electrode were included in the final analysis in order to allow meaningful comparisons between groups independently of electrode length.

4.2.2. Demographics and preoperative measures

The final cohort of 30 patients was comprised of 11 controls, 10 oral and nine transtympanic patients. The mean age was 65 years (SD 11.6) with 14 males and 16 females. The mean duration of deafness was 26.2 years (SD 15.9). There were no significant differences between the three groups in terms of their preoperative SSQ (Speech Spatial and Qualities of Hearing questionnaire) and HISQUI (Hearing Implant Sound Quality Index) scores, indicating similar perceived sound quality and listening abilities (Table 4.7).

The mean age for the individual groups was 65 years (control), 64.3 years (oral) and 64.9 years (transtympanic). These were not statistically different. There were slightly more females in the oral group and more males in the transtympanic group, although Fisher's exact test showed no statistical differences between the groups ($p>0.05$). The aetiologies of hearing loss were similar across all three groups with some exceptions. The transtympanic group had the highest proportion of noise-induced hearing loss (four out of nine subjects) and included one patient with autoimmune deafness and one patient with Marfan's syndrome. There were three diabetic patients in the control group and two diabetic patients in the transtympanic group, but none in the oral group, which had one patient with ototoxicity-related deafness.

The mean duration of hearing loss was 21.3 years (SD 11.1) in the control group, 28.9 years (SD 20.5) in the oral group and 29.2 years (SD 15.7) in the transtympanic group. This also did not differ significantly. The ear to be implanted was similar amongst all groups, although the transtympanic group had the highest proportion of right ears implanted (66%) and the control group had the highest proportion of left ears implanted (64%). However, the side of surgery performed did not differ statistically between groups. All surgeons who operated were right handed. The type of speech processor used did not differ significantly between the groups (Table 4.7).

The preoperative speech discrimination and audiometric results are shown in Table 4.8. The mean preoperative AF-PTA and LF-PTA did not differ statistically between groups. Similarly, there were no significant differences between the groups for all measures of speech discrimination ($p>0.05$).

Table 4.6 Demographics of patients excluded from the study

Group	n	Mean age in years	M: F ratio	Aetiology	Mean duration of deafness in years	Implanted ear (%)	Electrode types	Speech processor
Excluded from study	8	61 (20.3)	3: 5	Idiopathic – 3 Ear infections – 1 Mitochondrial -1 Familial – 1 Familial / noise induced – 1 Meniere’s -1	22.6 (14.7)	Right 50% Left 50%	28mm – 7 24mm -1	Opus II -5 Opus II/ rondo – 3
Excluded due to 31mm electrode	3	63.4 (8.0)	2:1	Idiopathic – 1 Meniere’s – 1 Noise induced - 1	23.3 (11.5)	Right 100%	31mm -3	Opus II -2 Rondo - 1
Excluded due to 24mm electrode	3	58.3 (10.8)	2:1	Idiopathic – 3	23.7 (17.0)	Right 66% Left 33%	24mm - 3	Opus II – 2 Duet 2 - 1

The numbers in brackets indicate the SD

Table 4.7 Demographics of patients included in the final cohort

Group	n	Mean age in years	M: F ratio	Aetiology	Mean duration of deafness in years	Implanted ear (%)	Electrode types	Speech processor
Control	11	65 (9.7)	5:6	Idiopathic – 7 Meniere’s – 1 Noise induced – 2 Familial – 1 (Diabetes – 3)	21.3 (11.1)	Right 36% Left 64%	28mm only	Opus II – 3 Opus II/ Rondo - 6 Rondo -2
Oral	10	64.3 (14.9)	3: 7	Idiopathic – 4 Familial – 2 Meniere’s -2 Noise induced -1 Ototoxicity – 1	28.9 (20.5)	Right 60% Left 40%	28mm – 10	Opus II - 6 Opus II/ Rondo - 2 Rondo - 2
Transtympanic	9	64.9 (11.1)	6:3	Noise induced – 4 Marfan’s – 1 Autoimmune – 1 Idiopathic – 2 Familial – 1 (Diabetes – 2)	29.2 (15.7)	Right 66% Left 33%	28mm only	Opus II – 4 Opus II/ Rondo - 5

The numbers in brackets indicate the SD

Table 4.8 Preoperative mean hearing and speech discrimination scores for all three groups

Group	Mean AF-PTA dB	Mean LF-PTA dB	CNC words (%)	AZBio in quiet % – best aided	HINT (Q) – best aided at 60dB SPL	HISQUI	SSQ
Control n=11	83.1 (12.3)	66.7 (7.8)	28 (14.1)	27.5 (20.4)	51.1 (17.8)	81.9 (35.5)	S= 2.56 (2.35) S= 3.03 (2.28) Q= 3.44 (2.17)
Oral n=10	81.8 (9.7)	68.5 (8.5)	31.6 (16.2)	27.3 (19.7)	47.3 (29.2)	83.9 (20.9)	S= 2.07 (1.25) S= 2.06 (0.94) Q= 2.94 (1.01)
Transtympanic n=9	79.1 (4.2)	61.9 (11.4)	34.8 (23)	32.2 (24)	49.9 (30.7)	73.3 (19.7)	S= 2.23 (0.90) S= 2.53 (0.90) Q= 3.32 (0.91)

The numbers in brackets indicate the SD

4.2.3. Treatment outcomes

All oral group patients completed their prescribed dose of prednisolone. Commonly reported side effects included increased appetite, insomnia and gastro-esophageal reflux which was not a reason to discontinue the steroid course. One patient from the transtympanic group was found to have a 5% marginal tympanic membrane perforation 2 weeks after surgery, but which closed just prior to CI activation. The site of perforation was not consistent with the transtympanic injection site scar, which was visible and in a separate location. The cause of the perforation was thought to be due to inadvertent injury to the tympanic annulus during the facial recess exposure with the drill. The patient was treated conservatively with ciprofloxacin eardrops and the perforation resolved spontaneously within 1 month.

4.2.4. Pure tone audiometry and speech outcomes

The greatest deterioration in PTA occurred between the preoperative period and 1 week after implant activation. The changes in PTA for both measures (AF-PTA and LF-PTA) are outlined in Table 4.9. Over the first 3 months on repeated measures ANOVA, the transtympanic group had a significantly lower AF-PTA compared to the other two groups ANOVA ($F=3.54$, $df(2,91,36.34)$, $p=.025$, Greenhouse Geisser correction). This was also seen when the LF-PTA measure was used ($F(2,26)=3.285$, $p=0.05$). At 12 months, the transtympanic group continued to have a better AF-PTA compared to the other two groups ($F(3.63, 4.85)=2.547$, $p=0.04$, Greenhouse Geisser correction). This was not seen in the LF-PTA (Figure 4.5).

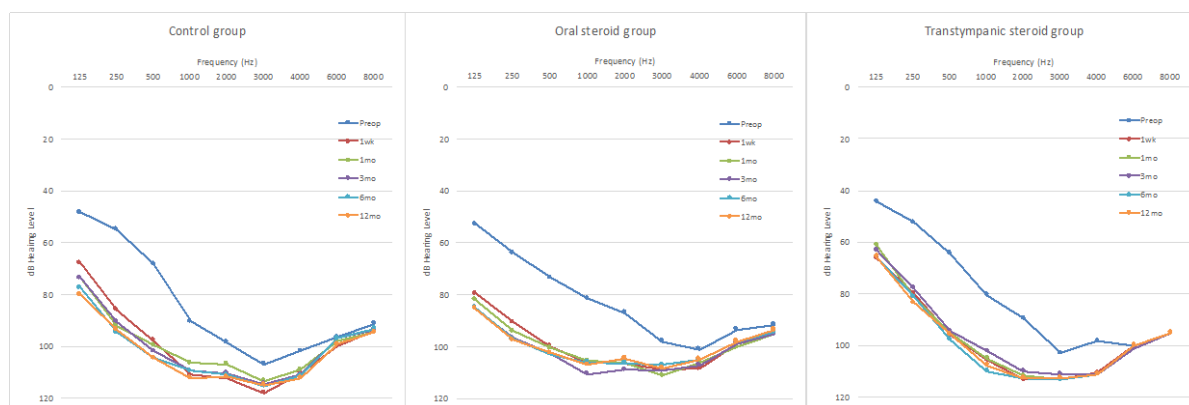
Table 4.9 Mean PTA and hearing preservation rate in all three groups over all time points

Hearing measure	Treatment groups	Time post-activation				
		1 week	1 month	3 months	6 months	12 months
All frequency AF PTA (dB)	Control	100.3 (10.3)	100.4 (8.7)	102.5 (7.6)	103.5 (8.1)	104.4 (6.2)
	Oral	100.4 (8.7)	100.5 (9)	102.8 (7.8)	101.4 (8.8)	100 (10.9)
	Transtympanic	98.6 (7.9)	97.6 (9.5)	95.6 (10.6)	99.2 (10.3)	99 (9.5)
Low frequency LF PTA (dB)	Control	91.1 (12)	92.9 (9.1)	94.2 (9.5)	96.5 (9.5)	97.6 (10.2)
	Oral	94.4 (9.7)	95.5 (8.7)	98.9 (7.2)	97.8 (8.8)	98.1 (9.9)
	Transtympanic	86.9 (13.7)	85.6 (16.3)	85 (16.7)	88.6 (16.6)	99 (14.3)
Hearing preservation rate (%S)	Control	39.4 (26.4)	37.9 (25)	32.5 (20.2)	28.1 (21.6)	25.9 (22.1)
	Oral	35.9 (28.2)	32.6 (26.6)	25.6 (22.7)	29.8 (24.6)	27.8 (30.2)
	Transtympanic	35.2 (22.7)	37.3 (27)	42.9 (31.5)	30.9 (27.5)	32.8 (25.1)

The numbers in brackets indicate the SD

The numbers in bold indicate a statistically significant difference on ANOVA ($p < 0.05$)

Figure 4.5 Mean pure tone thresholds at each frequency for the control, oral steroid and transtympanic groups over 12 months



The hearing preservation rate (S%) decreased in the control group from 39.4% at 1 week to 32.5% at 3 months. In the oral group, the S% also decreased from 35.9% at 1 week to 25.6% at 3 months. However, in the transtympanic group, the S% increased from 35.2% at 1 week to 42.9% at 3 months. This was significant compared to the other two groups on repeated measures ANOVA ($F=3.45$, $df(3,21,40.13)$, $p=.023$, Greenhouse Geisser correction). After 3 months, the transtympanic group S% approached that of the other two groups, but over 12 months there was no longer a significant difference between the three groups.

As expected, all measures of speech discrimination improved significantly in all three groups over the 12-month period of observation (Table 4.10). In the best aided situation, a significant improvement in CNC word scores over time was observed ($F=26.68$, $df(2, 48)$, $p<.0001$). Across all patients, there was also significant improvement in the best aided AZBio sentence scores in quiet and in noise over time ($F=26.85$, $df(2, 48)$, $p<0.001$, and $F=26.12$, $df(2, 48)$, $p<0.001$, respectively). When the CI only ear was tested, a significant increase in AZBio sentence scores in quiet was observed ($F=27.49$, $df(2, 48)$, $p<.0001$). However, there were no differences between the treatment groups over time.

Table 4.10 Mean word and sentence speech discrimination scores over all time points

Speech discrimination	Treatment groups	Time post-activation				
		1 week	1 month	3 months	6 months	12 months
CNC word score %	Control	31.1 (22.3)	44 (20.6)	55.6 (24.8)	58.6 (18.7)	61.2 (14.5)
	Oral	36.8 (13.9)	58.9 (15.4)	57.7 (13.6)	59.4 (16.2)	59.6 (11.7)
	Transtympanic	41.8 (29.3)	49.8 (30.8)	60.1 (26.4)	61.8 (25.2)	63.2 (15.7)
AZBio sentence score in quiet, best aided (%)	Control	37.3 (23.3)	53.5 (25.3)	64.7 (22.6)	67.9 (23.6)	68.1 (19.5)
	Oral	40.6 (19.3)	52.7 (25.8)	60.1 (22.7)	62.3 (19.5)	62.3 (21.2)
	Transtympanic	55.5 (33.4)	60.8 (29.2)	69.8 (32.1)	65 (27.2)	72 (32.2)
AZBio sentence score in quiet, implanted ear only (%)	Control	11.1 (15.1)	33.1 (32.2)	41.7 (29.7)	49 (29.1)	54 (29.2)
	Oral	24.8 (23.1)	45.8 (27.6)	43.7 (27.3)	49.4 (24.1)	54.6 (27.3)
	Transtympanic	34.9 (36.5)	46.5 (31.2)	56.3 (31.9)	46.4 (35.2)	54.2 (32.8)

The numbers in brackets indicate the SD

4.2.5. Discussion

The results of this study demonstrate that in addition to dexamethasone on induction and topically just prior to surgery, a single, preoperative transtympanic steroid administration was associated with a lower PTA and a higher rate of hearing preservation following CI activation. The reasons for this are unclear and seem at first glance to be counterintuitive. However, a single preoperative application of steroid has been shown to affect electrode impedance long after electrode insertion. Whilst reduced impedance does not necessarily translate into improved hearing, it does demonstrate that physiological changes occur at the level of the electrode long after steroid delivery. De Ceulaer et al. showed that electrode impedance was significantly lower up to 12 months

after surgery for patients who received intratympanic triamcinolone just prior to electrode insertion compared to control patients (De Ceulaer et al., 2003). Paasche et al. also assessed the use of triamcinolone injected into the cochlea just prior to electrode insertion and found not only lower impedances after 3 months compared to the control group but also up to 3 years, indicating that long-term effects are possible (Paasche et al., 2009). In a randomised controlled trial, Enticott et al. found lower impedances in the mid portion of the electrode in patients who had received a round window application of methylprednisolone. This was detectable between 2 to 9 months after surgery (Enticott et al., 2011).

The standard CI protocol at our institution involved the use of dexamethasone upon anaesthetic induction and just prior to opening the RW membrane. The use of steroids at these time points may have masked the observed effect from preoperative steroids. Due to ethical considerations, we were also unable to completely deny our control subjects all forms of intraoperative steroids, given there is some evidence in the literature for its use. Moreover, we could not exclude the contribution of intrinsic steroid production due to surgical stress. this may have offered some protection at the time of surgery. However, all three groups received the same intraoperative steroid treatment and the study was designed to assess the addition of preoperative transtympanic steroids, which was the main difference from the control group.

A patient's lead surgeon was not considered a potential confounder because there was no significant difference between the contribution of each surgeon in each treatment

group. The surgery was also performed at a university tertiary centre with involvement of the resident, staff surgeon and fellow. Residents and fellows differed in their ability to insert the electrode under supervision. As a result, we could not completely exclude differing surgical practices as a confounder especially since this was not a single surgeon experience. Whilst soft surgical techniques are practiced routinely at our institution, this was also not specifically prescribed. Factors such as insertion speed or the way the RW membrane was opened may have varied from surgeon to surgeon and therefore may be additional confounders. A mixture of lead surgeons in each group has reduced but not eliminated this factor.

As expected, all CI patients showed gradual and significant improvement in speech discrimination scores over time. However, this was irrespective of whether steroids were administered preoperatively. The absence of any significant differences in speech discrimination scores between treatment groups may have been due to the small patient numbers in each group. Despite this, significant differences were seen in the PTA and in the hearing preservation rate after implant activation. Future studies should take this into consideration and ensure that similar types of electrodes are used in order to avoid confounding factors.

The largest decline in hearing occurred within the first month after surgery, similar to results reported by Cho et al. in their cohort of patients (Cho et al., 2016). In their study and as also observed here, there is a more gradual decline in hearing following this initial

decline. This suggests the critical period during which preoperative steroids may be most beneficial is within the immediate postoperative period. The gradual decline in pure tone thresholds seen in our cohort of patients after CI has been reported previously by several authors. Santa-Maria et al., observed a progressive hearing loss over 24 months following electric-acoustic stimulation surgery with a 24mm electrode (Santa Maria et al., 2013). In a series of 127 full-length CI recipients, Cosetti et al. reported a hearing preservation rate of just under 30% (Cosetti et al., 2013). In patients with a short 10mm electrode, Gantz et al. showed that over 30% of subjects had more than a 30dB deterioration in their PTA up to 3 years post-CI (Gantz et al., 2009). However, in each of these three studies there were no correlations between speech scores and declining hearing levels. This raises the question of whether hearing preservation should be one of the aims of CI surgery. In our study we found that despite decreasing PTA levels and improved rates of hearing preservation in the transtympanic group, there was no effect on speech performance. Despite the lack of effect on open set speech performance, we propose the benefit on hearing observed in our study should not be dismissed as it could reflect other aspects of cochlear function that were not assessed. For example, various electrophysiological parameters such as the ECAP, electrode impedance, cochlear microphonic and auditory nerve neurophilic may show changes. Further studies are required to determine whether these parameters are altered by the use of steroids.

Low frequency hearing preservation has already been shown to be important for listening in challenging situations (Gifford et al., 2013) and for sound localisation and music appreciation (von Ilberg et al., 1999). More challenging tests of speech

recognition may have uncovered differences between the experimental groups. Although the subjects in our study were not EAS candidates, EAS patients with greater residual hearing may have gained more benefit when there is greater preservation of low frequency hearing.

In our study, low frequency hearing appeared to be preferentially preserved in the transtympanic group in the short-term. However, there is a known gradient of declining steroid concentration from the base to the apex of the cochlea following transtympanic injection, thus resulting in a small amount of steroid reaching the apex where low frequencies are coded (Plontke et al., 2008). In addition, locally applied steroids to the round window are no longer measurable after 24 hours of exposure (Chang et al., 2009). One possible explanation to reconcile these phenomena with our result of short-term low frequency hearing preservation is that despite the small amount of steroid reaching the apex, there are sufficient genomic steroid effects because of the prolonged time of exposure. The observation of significant differences in hearing results when all frequencies were averaged also supports the notion that concentration gradients alone may not be the only mechanism of action.

The lack of a clear benefit from oral prednisolone may have been due to several factors. Compliance with the drug being taken by patients was only assessed by self-reporting and minor side effects may have led to surreptitious dose reduction or avoidance. Patients were required to take multiple tablets to make up the prescribed dose and this may have further increased the risk of non-compliance. In addition, biases may have

occurred due to the exclusion of patients with diabetes who could not take systemic steroids because of adverse effects on blood sugar levels. For other types of inner ear pathologies such as sudden sensorineural hearing loss, evidence for benefit from oral steroids has been equivocal (Wei et al., 2013). Oral steroids have also been compared to transtympanic steroids for treating sudden hearing loss and found to be less beneficial (Filipo et al., 2014). Perhaps alternative routes for systemic drug delivery such as intravenous or intramuscular could be pursued. The transtympanic route, however, is not without limitations. It requires the use of a microscope, local anaesthesia and cooperation of the patient. It can result in discomfort and vertigo. It also requires that patients attend prior to surgery, which may be impractical or inconvenient. The transtympanic route is also affected by middle ear clearance through the Eustachian tube and local RW factors that can result in a wide variability of concentrations within the perilymph (Bird et al., 2011). These may be overcome by using higher concentrations of steroid, improving access to the RW niche such as through endoscopic means, or by repeated or sustained drug delivery techniques (Salt et al., 2011). Further studies are needed to assess the potential benefit of these different options for steroid delivery.

4.2.6. Conclusion

This study demonstrates potential benefits from application of preoperative transtympanic dexamethasone. In addition to our routine intraoperative regime, an additional single transtympanic shot of dexamethasone given 24 hours preoperatively gave a better hearing preservation rate at 3 months and a better pure tone average at 3 months and a year at all frequencies. However, low tone frequency PTA was only

significantly improved transiently at 3 months and was not sustained by the end of the study, nor was there a significant hearing preservation rate.

Further studies and longer-term follow up are required to more fully evaluate the potential benefits from this technique.

5. General discussion

5.1. Summary of results

The research findings presented here and obtained using an animal model and CI patients suggest that extended preoperative steroids have beneficial effects in reducing the hearing loss associated with CI.

In the animal study, we used a well-accepted model for CI that mimicked the current trend towards atraumatic electrode insertion. These were inserted deeper than previously published studies in order to evaluate effect of steroids on electrode-related trauma. We utilised systemic steroids from 24 hours to 5 days prior to surgery. Despite the expected low penetration into the cochlear compared to transtympanic or intracochlear routes, we found beneficial effects on hearing, tissue fibrosis and SGN density. These effects were most pronounced in the basal turn of the cochlear, in a similar manner to the round window application of steroids. There were no significant differences in hearing outcomes between a single dose and a long course of steroids. However, the longer course of steroids produced more significant histopathological changes.

In the human study, the transtympanic steroid group showed better preserved low frequency hearing and better speech discrimination scores in the implanted ear. The systemic oral steroid group did not show any real benefits compared to the control group.

5.2. Overall limitations

Several weaknesses were inherent to each of the animal and human studies. Fundamentally, the relevance of the animal model to patients can be questioned. Normal hearing guinea pigs with arguably a greater tolerance for inner ear trauma were used, whereas the typical human CI candidate may have a variety of hearing loss aetiologies, varying degrees of SGN survival and hair cell loss.

In the animal study, we did not perform other objective measures of hearing such as otoacoustic emissions which could then have been correlated with hair cell morphology. In addition, the method used to visually inspect the ABR threshold is arguably subjective, although this can be reduced by using two reviewers or by determining a minimum voltage required for the presence of wave V of the ABR. We also did not perform any electrophysiological measurements to allow more direct comparisons with the human side of the study. Measures such as ECAP slope would be useful to compare with SGN density and morphology, while ECAP threshold and impedance can be correlated with the degree of electrode fibrosis.

Intraperitoneal dexamethasone could induce a specific type of systemic immune response which may alter the degree of hearing loss post-CI surgery (Souter et al., 2012). To investigate this issue further, perilymph would need to be sampled at the time of cochleostomy, similar to the study by Parnes and Sun, 1999. However, sampling of perilymph may add trauma to the inner ear and is against the principles of “soft surgery” (Lenhardt et al., 1993). Another issue which was not addressed is the effect of intraperitoneal steroids on the unimplanted ear. No literature reporting the effect of

intraperitoneal dexamethasone on ototoxicity could be found, although its safety profile has been demonstrated via other routes (Tanaka et al., 1994). The lack of hearing threshold data on the contralateral ear meant we could not completely assess the safety profile of intraperitoneal dexamethasone in our study. We did not observe any side effects of systemic steroids in these animals, although it should be noted the sample size and duration of follow up was limited. Because we only implanted the electrode and not the receiver stimulator, no comment could be made regarding systemic steroid effects such as implant retention rates, foreign body reaction or risk of biofilm formation.

The human study also had several limitations. The total number of patients in each group was relatively small. Despite recruiting from the largest adult CI centre in Canada, the number of patients who met the audiological inclusion criteria and who were willing to undergo oral or transtympanic steroids was small. A true blinded randomised controlled trial could not be undertaken because the small number of patients prevented the use of a placebo. Limiting the steroid intervention group to one modality would have increased the power of the study.

Whilst all three groups received the same steroids on induction and just prior to electrode insertion, thereby eliminating this as a confounder, it does limit the study's applicability. One cannot assume that preoperative transtympanic steroids *alone* would have the same positive effects as what has been shown in this study. It is therefore possible that the benefits of preoperative dexamethasone are only measurable when intraoperative steroids are given in concurrently.

Despite this limitation, many surgeons utilise steroids intraoperatively just prior to or after electrode insertion because this is most convenient and perhaps the short time of exposure renders most intraoperative steroid effects the same regardless of dosing and steroid type. The addition of preoperative steroids the day before surgery is therefore a novel option that may have practical merit in the day to day management of CI patients in centres where intraoperative steroid use is already routine.

5.3. Systemic preoperative steroids in humans versus animals

There are several important points to note when comparing animal and human subjects receiving systemic steroids. A benefit from systemic preoperative steroids was found in the animal study, but not in the human trial subjects. There may be a number of reasons for this. Ignoring the pharmacokinetics of the oral route (which is subject to 1st pass liver effects) and of the intraperitoneal route (which bypasses this to a large extent) in dose comparison studies, 2mg/kg of dexamethasone in guinea pigs is equivalent to 0.434mg/kg in an adult, or 30mg of dexamethasone in the average 70kg adult. This is equivalent in potency to 60mg of prednisolone (Tanaka et al., 1994).

This dose is similar to the oral dose used in the majority of our adult subjects weighing more than 50kg, with a maximum dose ceiling of 60mg based on 1mg/kg. Oral prednisolone was chosen because it is the oral steroid of choice in our institution for the treatment of sudden sensorineural hearing loss. This meant we were familiar with its side effect profile and the medication was readily available. However, there is a

well-known difference in potency, with dexamethasone having 6.25-37.5 times greater anti-inflammatory activity (Tanaka et al., 1994). Since both drugs have a similar plasma half-life profile (120 to 300 minutes for prednisolone and 150-270min for dexamethasone), the human adult would need to ingest between 187.5mg to 1,125mg of oral prednisolone in order to obtain a similar anti-inflammatory activity. Potency is defined here as the ability of the steroid to upregulate genes responsive to glucocorticoid-induced transcription (Tanaka et al., 1994).

5.4. Intravenous versus intraperitoneal route

The intraperitoneal route is easier to administer than the intravenous route. The dose chosen was based upon the systemic dose trials of Connolly et al., who found benefits with 2mg/kg dexamethasone instead of 0.2mg/kg given intravenously (Connolly et al., 2011). Liu et al. examined the pharmacokinetics of intraperitoneal versus transtympanic dexamethasone in guinea pigs (Liu et al., 2006). In their study, the perilymph concentration of dexamethasone was measured following intraperitoneal injection of 0.5% dexamethasone (equal to 4mg/kg) and 150uL intratympanically. Whilst the authors concluded that both routes achieved the same concentration at 30 minutes, it should be noted the intraperitoneal route results in a higher maximum concentration and takes 30 minutes to achieve this, whereas the transtympanic route reaches peak concentration within 10 minutes before steadily declining. The calculated half-life of dexamethasone elimination from the perilymph was similar in both cases (2.9 hours), with the drug being undetectable after 6 hours.

Parnes et al. did not find evidence of perilymph steroid penetration after oral dexamethasone, but did find evidence after intravenous dosing at 8mg/kg (Parnes et al., 1999). This was double the intraperitoneal dose used by Liu et al (Liu et al., 2006). The time to reach maximal concentration in the study by Parnes et al. was 1 hour after intravenous injection, compared to 2 hours after intraperitoneal injection. The maximal concentration levels found by these workers after intravenous dosing were significantly lower than in Liu et al.'s study of intraperitoneal dosing at lower drug doses of 4mg/kg versus 8mg/kg (Parnes et al., 1999; Liu et al., 2006).

In other studies, Bird et al. measured the perilymph concentration 90 minutes after giving intravenous dexamethasone at a dose of 0.17mg/kg and found concentrations 88-fold lower compared to transtympanic dexamethasone (Bird et al., 2011). In an animal study, Tobita et al. found the peak concentration after giving 100mg/kg of systemic prednisolone occurred at 1 hour (Tobita et al., 2002).

From the above studies, it is clear that systemic dexamethasone results in measurable levels of the drug in the perilymph due to steroid crossing the blood brain barrier. It is also apparent that intraperitoneal injection produces a very different time concentration curve. The slower time to peak (2 hours versus 1 hour) could allow greater time for steroid to equilibrate across the blood labyrinthine barrier, thus resulting in higher concentrations in the perilymph at a lower dose. This factor should be considered when giving intravenous doses in human studies to study steroid effects post-CI surgery. This

issue could potentially be overcome by using a very high intravenous dose or by using a systemic depot preparation of steroids, for example intramuscularly.

The ideal concentration in order to achieve measurable effects is likely to be different between animals and humans, thus making it difficult to give a recommendation. The ideal preoperative intravenous dose of dexamethasone in humans is probably $>0.4\text{mg/kg}$, but this will be difficult to confirm because periodic sampling of perilymph in humans is impractical.

One suggestion for non-invasive monitoring of the response of the inner ear to systemic steroids is to measure expression of the glucocorticoid receptor in peripheral blood mononuclear cells (Lu et al., 2013). In this study in guinea pigs, 10mg/kg of intraperitoneal dexamethasone was administered for 7 days before measuring glucocorticoid receptor mRNA and protein levels in blood and in the cochlea. Both the mRNA and receptor protein levels in the cochlea correlated well with the same measure in peripheral leucocytes ($r=0.8$). Administration of dexamethasone resulted in an increase in both mRNA and receptor levels compared to a control group. For short-term systemic steroids, peripheral analysis may therefore have a role in determining the response of the inner ear. Whilst these results are encouraging, they do not take into account if there is a dose response curve, the applicability to intravenous dosing, or the role of mineralocorticoids.

5.5. Systemic versus transtympanic route

The most recent animal study to examine the longer-term outcomes of systemic and transtympanic steroids was by Lee et al. This paper extended the results of Connolly et al. who found that 2mg/kg of intravenous dexamethasone 1 hour prior to surgery resulted in less hearing loss at 1 month and a trend towards less fibrosis (Lee et al., 2013; Connolly et al., 2011). In Lee et al.'s paper, 2mg/kg of intravenous dexamethasone 1 hour prior to surgery was compared to 20% RW dexamethasone for 30 minutes and to 2% dexamethasone for 2 hours. Both the systemic and the 2% for 2 hour groups had lower thresholds compared to the control group for up to 3 months, suggesting both options are equally effective. There was no difference between the two transtympanic concentrations after 3 months. However, the systemic group at 3 months had lower OHC counts but less basal turn fibrosis. This was proposed to be due to different effects of systemic and topical steroid applications on activation of the cellular immune response (Tailor et al., 1994). Indeed, Souter et al. showed that altering the peripheral immune response may change the cochlear response to trauma (Souter et al., 2012).

For the transtympanic route, there is clearly evidence both from animal studies and in humans that preoperative steroids are beneficial. Several of the factors which affect this response have been characterised and include concentration, time of application and route of administration (transtympanic, intracochlear, depot).

It is clear than preoperative systemic steroids are beneficial, at least in animals. However, the factors which modulate this are less clear. For the intravenous route, the larger the dose given, the higher the perilymph concentration. However, with the

intraperitoneal route the slower rise to peak concentration may be more beneficial. With either systemic route, the site of maximal steroid uptake appears to be concentrated at the base and because of potential effects on the peripheral immune system, the response of the inner may also be different (Souter et al., 2012; Lu et al., 2013; Lee et al., 2013). One unresolved issue is whether there is a role for systemic steroids in humans. Based upon the evidence presented to date, such a role may well exist but the potential benefit needs to be weighed against the risk of systemic side effects. To achieve a high enough concentration in the inner ear with the shortest possible peripheral exposure, a single dose of steroids might be the mode of choice. The alternative is to use a short-term depot preparation of systemic steroids (e.g. intramuscular) which avoids the side effects of oral dosing.

5.6. Long-term results – can hearing loss after implantation be influenced by steroids?

There is growing concern within CI research centres of the long-term outcomes following hearing preservation surgery. Whilst the ability to preserve hearing is possible and can be optimised, long-term hearing loss has been demonstrated by several authors. Santa-Maria et al. showed there is progressive hearing loss over 24 months following electric-acoustic stimulation surgery with a 24mm electrode, but this did not lead to concomitant deterioration in speech performance (Santa Maria et al., 2013). In their study, 12mg of dexamethasone was used on induction followed by methylprednisolone just prior to cochleostomy. After 24 months, just under half the patients were able to wear a hearing aid in conjunction with their CI in the same year, from nearly all patients

at the beginning of the study. In a series of 127 full length CI recipients, Cosetti et al. showed a hearing preservation rate of just under 30%, with no correlation between the PTA and speech discrimination (Cosetti et al., 2013). Gantz et al. showed that even in patients with a 10mm electrode, more than 30% of subjects up to 3 years post-surgery had more than a 30dB deterioration in their PTA (Gantz et al., 2009). As with the other two studies (Santa Maria et al., 2013; Cosetti et al., 2013), there was no correlation between the degree of hearing preservation and CNC word scores.. Given that other studies have reported improved hearing preservation, when residual hearing does inevitably deteriorate over time below a threshold level it is no longer associated with speech performance. Concomitantly, the CI can provide appropriate compensation.

Several factors have been associated with delayed hearing loss at 1 year, including older age at implantation, gender, and noise-induced hearing loss aetiology (Kopelovich et al., 2014). The mechanism of this delayed hearing loss is varied and several possibilities have been suggested. These can be categorised into tissue response to the electrode, immune or inflammatory mediated injury, vascular injury and even electrical stimulation. Choi and Oghalai showed the degree of fibrosis in the basal turn of the cochlear within the scala tympani reduces the mechanical tuning at the apex of the cochlea, with greater degrees of fibrosis affecting a wider range of frequencies (Choi and Oghalai, 2005). O’Leary et al. showed that the level of fibrosis within the basal turn is correlated with hearing loss at 8kHz and 16kHz, but not at 32kHz where osseous spiral lamina fracture was a factor (O’Leary et al., 2013). Animals with more severe hearing loss but less fibrosis were observed, indicating the well-known fibrotic reaction around

electrodes (Nadol and Eddington, 2006) is not the only reason for hearing loss. Eshraghi et al. showed there are molecular mechanisms which occur following the initial surgical trauma which lead to apoptosis (Eshraghi et al., 2013). Wright and Roland showed in a temporal bone study that draining venules along the lateral wall have either minimal or no bony coverage, making them susceptible to trauma (Wright and Roland, 2013). This could then lead to vascular trauma to the stria vascularis which then affects the endocochlear potential. Tanaka et al. compared guinea pigs who had been implanted and then subjected to either combined electrical and acoustic stimulation or to no stimulation (Tanaka et al., 2014). The combined stimulation group had greater hearing loss at low frequency (1kHz) after 10 weeks of observation. There was also a correlation between hearing loss and stria vessel area and density, as well as hearing loss in the absence of any macroscopic evidence of hair cell loss. The latter is probably due to the low trauma of the model, but indicates that many effects of hearing loss are not visible by microscopy, confirming other studies (Kang et al., 2010). These findings also suggest that electrical stimulation with acoustic stimulation may contribute to hearing loss, but to a lesser degree than vascular injury. The evidence for electrical stimulation from the implant causing acoustic hearing is variable, with evidence for and against this (Coco et al., 2007).

Some of these mechanisms may be influenced by steroid administration. For example, steroids have been shown to increase the blood flow to the inner ear, potentially overcoming the reduction in stria vessel area or density (Shirwany et al., 1998). Steroids may also act by reducing the elevated permeability of the stria vascularis following

vibrational trauma (Hashimoto et al., 2006). It is well known that steroids reduce the degree of fibrosis, which according to Choi and Oghalai, may have effects distant to the site of electrode insertion (Choi and Oghalai, 2005). Together with the known genomic effects on inner ear cells and in reducing apoptosis, steroids may affect long-term hearing loss relating to CI in multiple ways and which may not be visible histologically. Further studies are clearly warranted to explore this issue further.

5.7. Steroid eluting electrodes

An emerging area of research which is complementary to our study is the development of drug eluting steroids. Drug eluting implants in the form of stents or pacemaker leads have been investigated for some time in cardiology; however, drug eluting CI electrodes are relatively new. There are currently no published human trials on steroid eluting electrodes. The degree of steroid impregnation can be controlled to alter its pharmacokinetics (Dinh et al., 2008, Farahmand et al., 2010). In a similar fashion to free steroids, drug eluting electrodes have been shown to influence the apoptosis pathway in a TNF- α model of hearing loss, with upregulation of *Bcl-2* and *Bcl-XL* genes, downregulation of *Bax* genes and preservation of outer and IHCs (Dinh et al., 2008). Over a 2-week period, drug eluting electrodes were shown to reduce the inflammatory cell infiltrate by inhibiting the fibroblast, macrophage and giant cell reaction, as well as reducing neovascularisation (Farhadi et al., 2010).

In a recent guinea pig study, dexamethasone at three concentrations up to 24% w/v was painted onto electrodes and implanted for 3 months (Stathopoulos et al., 2014a). No

significant differences compared to controls were observed in terms of hearing loss, fibrosis or SGN density, although there were trends towards favourable measures in the steroid groups. Cumulatively, there was an association between basal turn SGN density and hearing at 32kHz. No increase in the risk of meningitis was observed (Stathopoulos et al., 2014b).

Drug eluting steroids were reported to improve hearing thresholds in an animal study (Jolly et al., 2010). Drug eluting implant grade silicone with 2% or 10% dexamethasone was inserted up to 4mm into guinea pig cochleae. Compared to a control group, significantly less hearing loss was observed for up to 6 months, although the study did not detail what frequencies were tested other than “mid to high frequencies”. A similar result was shown with dexamethasone reservoir tubes implanted into guinea pigs.

Lacking in the current literature, however, is firm evidence showing improvements in hearing thresholds, SGN density and electrophysiology. Continued research will hopefully shed more light in these areas. At present, there is evidence for the use of preoperative steroids and this highlights the importance of pre-empting the cascade of events that occurs once the inner ear is opened and the electrode inserted. This may be more important than attempting to rescue the inner ear after electrode insertion when inevitable tissue responses have already started to occur. Studies that investigate a combination of both preoperative and drug eluting steroids should also prove worthwhile.

5.8. Consensus statements on steroid use in cochlear implantation and current clinical implications

At the time of writing this thesis, there was no consensus statement on the use of steroids in hearing preservation CI. Despite recent international meetings to discuss this topic, there remains considerable disagreement between experts on many aspects of steroid use. Most experts agree there is substantial evidence from animal studies (Proceedings from Round Table Discussion at Asia Pacific Symposium on Cochlear Implantation, Hyderabad 2013 and International Conference on Cochlear Implants and Related Sciences, Munich 2014).

There are several clinical implications from this work. Given the expanding criteria for CI and the inclusion of patients with greater degrees of residual hearing, we argue there should be a role for extended preoperative steroids in routine clinical practice. The outstanding issues involve the route to be used and in which patients this should apply. Until there is more evidence for the role of systemic steroids in humans, our evidence suggests that preoperative steroids should be limited to the transtympanic route. Patients with the most residual hearing (for example, those who would qualify for electric-acoustic stimulation) who have more to lose (and more to gain) should perhaps be offered this option after appropriate counselling. Another group may be patients who are undergoing cochlear re-implantation and where maximum effort should be made to limit inner ear trauma.

Despite our findings of short-term hearing and speech improvements that diminish over time, the effect of accelerating the time taken to maximise hearing performance may be

beneficial in patients who are at risk of poor speech performance. The initial increase in performance may also serve to encourage and motivate patients. With regards to the reduction in impedances, this may lead to improved battery life which in turn may increase patient implant usage, improve satisfaction with the device and allow the development of totally implantable devices with highly efficient power usage. As mentioned, long-term effects on impedance suggest long-term effects on tissue fibrosis which could thus alter the natural history of progressive hearing loss late after CI.

The implications of our findings for hearing preservation CI are relevant and contemporary. Amoodi et al. showed that implantation of patients above the traditional criteria for CI (i.e. HINT score >60%) were nevertheless able to realise significant benefits (Amoodi et al., 2012). More recently, Carlson et al. reported the same finding in the paediatric population and concluded that lack of hearing aid benefit, rather than a specific residual speech performance, may be sufficient reason to implant (Carlson et al., 2015). Paediatric patients with a PTA >70dB were included in their study, suggesting that residual hearing is not just present in adults. Mick et al. recently showed that hearing preservation is possible even with long electrodes (Mick et al., 2014), whilst Usami et al. also showed that it is possible in shorter electrodes (Usami et al., 2014).

5.9. Future directions

There are many future directions for research in the field of preoperative steroids and CI. In future animal studies, electrophysiological parameters such as ECAP, ECOG and OAEs should be assessed in addition to ABR and histopathology. This would help to

bridge the gap between laboratory research and clinical application where electrophysiology is a powerful tool to assess the status of the inner ear. Better animal models to assess CI surgery could be developed in which for example there is a loss of hair cells, preservation of SGNs, and the presence of neurotrophins to better mimic the inner ear status of the typical adult CI candidate. Systemic preoperative steroids should also be compared with emerging new steroid delivery techniques such as drug elution, either alone or in combination (Jolly et al., 2010).

With regards to human studies, a single high dose systemic steroid trial or a short-term depot steroid study should be conducted to determine whether these give measurable benefits. An ongoing trial by the Melbourne group will investigate the role of a single high dose of preoperative steroids (O’Leary et al, personal communication). A larger series of transtympanic preoperative steroid treated patients should also be conducted to extend the findings of the current study. In addition to ECAP and Impedance measures, emerging new techniques such as the ECOG should be included which provide more sensitive measures of hair cell function and prediction of speech performance. Newer and more rapid methods of ECAP measurements should also be employed to improve the resolution at which changes in the inner ear electrophysiology can be detected.

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