CLINICAL STUDY PROTOCOL

Pharmacokinetics and safety of intratympanic adenosine receptor ligands

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Locality	**Christchurch**

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1. Background and Study Rationale

Medications have significant potential in the treatment of acquired hearing loss, but much remains unknown about how these drugs behave in the inner ear. In earlier work we demonstrated human perilymph sampling for locally delivered methylprednisolone (Bird et al. 2007). Our animal modelling builds on earlier work by others (Ford et al. 1997) and has identified adenosine receptors as another possible vector for cochlear protection (Vlajkovic et al. 2010, 2014; Chang et al. 2017). Adenosine is an endogenous neuromodulator and a cytoprotective substance released from tissues in response to stress (Fredholm 2007). This occurs through its action on four G protein-coupled receptors designated A1, A2a, A2b and A3 (Vlajkovic et al. 2009). Two ligands binding to the adenosine A2 receptor (ATAR) have demonstrated otoprotective effects.

Istradefylline, a A2AR antagonist, is used in Parkinson's disease and we have shown this reduces deafferentation of inner hair cells and improves the survival of afferent synapses after excitotoxic injury (Han B et al 2019). Cochlear injury which is mostly due to oxidative stress or glutamate excitotoxicity, such as with the chemotherapy agent cisplatin or noise induced injury, can be reduced by blocking the A2AR which then tips the balance of adenosine towards the A1R environment which is strongly otoprotective. It is theorised this could have benefit in cisplatin induced hearing loss and high frequency hearing loss associated with stapedectomy and other surgical procedures (Babbage et al 2017).

Regadenoson is A2AR agonist, commonly used as a cardiac stimulant in myocardial perfusion tests. Our group have also demonstrated that it suppresses cochlear inflammation in an animal model (Tan 2015). It is thought that it may have a role in preserving residual hearing during cochlear implantation.

Systemic side effects from these agents will be minimised if they are delivered via an intratympanic route (Liu et al. 2013). It is anticipated that the plasma concentrations of drug delivered transtympanically will be an order of magnitude less than the established safe serum levels for the established systemic uses of these drugs in Parkinson's disease and myocardial perfusion tests. Additionally, we will be administering these A2AR ligands while the patient is under general anaesthetic and will be having their cardiovascular system closely monitored by an anaesthetist.

Drugs delivered intratympanically must stay in contact with the round window membrane and annular ligament of the oval window for a long enough period to enable sufficient diffusion into the cochlea. Poloxamer 407 is a thermoreversible gel in which drugs may be suspended (Giuliano et al. 2018) and slowly released into the middle ear in the region of the round window membrane. The slow release should enable a more constant diffusion process across the membrane and more reliable concentrations within the cochlear fluids than would injection of a solution (Musazzi et al. 2018).

We aim to study the pharmacokinetic properties of both of these agents when delivered via a slow release intratympanic method. First, via in-vitro experiments to measure the drug elution over time from the gel. Then in patients undergoing surgery with routine exposure of the inner ear (translabyrinthine tumour removal or cochlear implantation) we will measure both the plasma and perilymph drug concentration at different time points to establish the safety of a transtympanic injection of these agents and help identify therapeutic concentrations of the drugs. These translational studies are critical to establish a safe and effective delivery system in preparation for clinical trials of efficacy.

1.1 Therapeutic agents

1.1.1 Istradefylline - The precise mechanism of action is unknown. Studies have demonstrated it to be an adenosine A2a receptor antagonist. It is used in Parkinsons disease as adjunctive treatment to levodopa/cardidopa and recommended dosing is 20-40mg daily orally. Istradefylline is completely metabolised primarily by CYP1A1 and

CYP3A4. The mean terminal half life is approximately 83 hours. Systemic side effects include dizziness, anorexia, insomnia, and constipation.

1.1.2 Regadenoson - Regadenoson is an adenosine derivative and selective A2a receptor agonist. Regadenoson rapidly increases coronary blood flow by inducing coronary vasodilation. It is used as a pharmacological stress agent for radionuclide myocardial perfusion imaging. It is renally excreted and the metabolism is unknown. Recommended dosing is 0.4mg (within 5ml saline) intravenously. The mean terminal half life is 2 hours. Due to its cardiac effects the systemic side effects include dyspnoea, headache, flushing, angina and nausea.

2. Study Objectives

Building on the background of our established human perilymph sampling methodology for transtympanically delivered methylprednisolone, we aim to extend this technique to adenosine A2a receptor ligands bound to a slow release gel. Specifically, we seek to perform both in-vitro and in-vivo trials to establish the safety and therapeutic concentrations of intratympanic Istradefylline and Regadenoson. These two studies will then provide the foundation for future clinical trials with these ligands to investigate the therapeutic benefit in humans on hearing and balance preservation.

3. Study Design

3.1. Overview of Study Design

The design of the first part of the study establishes the properties of drug release from the poloxamer gel for both Istradefylline and Regadenoson. While both agents have been polymerised to Poloxamer 407 in animal studies, there is no data on what the actual concentration of liberated drug is over time. This is important in humans because too low a concentration will be sub-therapeutic and too high a concentration risks stimulating other adenosine receptors which may alter the desired therapeutic effect.

The therapeutic agents will be sourced from their manufacturers: Istradefylline (Sigma), Poloxamer-407 gel (Sigma) and Regadenoson (Sapphire Bioscience). Based on prior animal studies, the gels will be prepared at 17% w/w in cold distilled water. Istradefylline 1 mM stock solution will be prepared in 40-50% Dimethyl Sulfoxide (DMSO) and then diluted down to 200 nM. Regadenoson will be similarly prepared with 2.5 mg of drug dissolved in 2 ml DMSO before being diluted to 1:100 to give a 1% DMSO concentration. The drug solutions will then be mixed with the 17% w/w poloxamer and placed on ice until fully dissolved. To model liberation of the drug from the gel in the approximately 1 mL volume of the human middle ear, 1 mL of thermoreversible gel will be warmed to body temperature (37 degrees Celsius). To approximate the fluid volume of the cochlea, 100 microlitres of saline will be placed on the gel and 20 microlitre aliquots taken at 30 minutes, 2 hours, 4 hours and 1 week and analysed by liquid chromatography - tandem mass spectrometry to establish the liberation of the drugs from the gel.

The design of the second part of the study involves adults who will already be having their inner ear exposed with translabyrinthine vestibular schwannoma removal (N= approximately 15), or cochlear implantation (N=approximately 20). We will establish the systemic safety by first performing this on patients undergoing translabyrinthine procedures

which are inherently longer and more closely monitored by the anaesthetist. We will then perform interim analysis after 6 translabyrinthine procedures for each drug. When we have demonstrated safe (low) systemic levels, we can then extend to include CI patients.

Following induction of anaesthesia, the drug-poloxamer gel will be injected transtympanically and applied over the round window membrane and the standard operation will proceed. Plasma samples will be taken at 30 minutes, 2 and 4 hours after placement of the gel. At the time of labyrinthectomy or electrode insertion, time since drug-gel injection will be noted and 20 microlitres of perilymph will be sampled from the round window using a Hamilton syringe. The perilymph will then sent for analysis using the liquid chromatography - tandem mass spectrometry detection method to ascertain the drug concentration within the perilymph of the cochlea basal turn, confirming delivery of drug. During the anaesthetic the anaesthetist monitors the patient for any signs of cardiac stress. For this study we aim to recruit 20 patients (N=10 each of Istradefylline and Regadenoson).

3.2. Study Endpoints

1. In vitro study: The rate of diffusion from the poloxamer gel into saline will be measured at separate time intervals in order to determine the liberation of drug from the poloxamer gel.

2. In vivo study:

    1. Establish the safety of the adenosine ligands topically in the middle ear. This will be through serial measurement of plasma concentrations and systemic (cardiovascular) effects

    2. Establish a middle ear delivery system of the adenosine ligands which is consistent

4. In-vitro Study procedure

4.1. Drug preparation

4.1.1. The therapeutic agents will be sourced from their manufacturers: Istradefylline (Sigma), Poloxamer-407 gel (Sigma) and Regadenoson (Sapphire Bioscience). A formulating pharmacist will make up the following. Based on prior animal studies, the gels will be prepared at 17% w/w in cold distilled water. Istradefylline 1 mM stock solution will be prepared in 40-50% Dimethyl Sulfoxide (DMSO) and then diluted down to 200 nM. Regadenoson will be similarly prepared with 2.5 mg of drug dissolved in 2 ml DMSO before being diluted to 1:100 to give a 1% DMSO concentration. The drug solutions will then be mixed with the 17% w/w poloxamer and placed on ice until fully dissolved. To model liberation of the drug from the gel in the approximately 1 mL volume of the human middle ear, 1 mL of thermoreversible gel will be warmed to body temperature (37 degrees Celsius)

4.2. Establishing rate of liberation of the drug from Gel

4.2.1. One (1)ml of the warmed drug will be placed in an Eppendorf tube. 100 micro litres of saline will be placed on it and the Eppendorf tube placed in a 37 degree water bath. At 30 minutes, 2 hours, 4 hours and 1 week 20 micolitres of saline will be removed by micropipette and sent for analysis using the liquid chromatography - tandem mass spectrometry detection method. After each 20 micro litre aliquot is removed it will be replaced by the same amount of saline to ensure the same concentrations.

5. In-vivo Study procedure

5.1 Recruitment

1. Patients who will undergo elective translabyrinthine resection of vestibular schwannoma at Christchurch Hospital or Cochlear Implantation at St George's Hospital will be approached by the surgeon, separate from the surgical consent process.

2. These patients will be given a verbal and written handout of the study procedure and will be consented (See appendix 1)

3. A detailed medical history will be taken during the admission process as well as routine biochemical assessment in order to identify exclusion criteria

4. Eligibility

a) Inclusion criteria

i. Patients undergoing elective translabyrithine resection of vestibular schwannoma or

ii. Patients undergoing Cochlear Implantation

b) Exclusion criteria

i. Unstable cardiac disease (Arrhythmias, recent MI/stent)

ii. Renal impairment (Creatinine >100 umol/L)

5.2 Detail of procedure

1. Prepared drug-poloxamer gel solution will be placed in a 1ml syringe with a 22G spinal needle attached

2. After the induction of general anaesthesia the WHO surgical safety checklist will be performed to ensure the correct procedure, site and side.

3. With an operating microscope the drug-poloxamer gel solution will be placed trans-tympanically through the antero-superior portion of the tympanic membrane over the round window.

4. The elective surgical procedure will then proceed as planned with a cortical mastoidectomy , posterior tympanotomy and exposure of the bony labyrinth.

5. Prior to the opening of the inner ear the middle ear will be washed out with 20 mL normal saline to ensure the complete removal of the drug-poloxomer gel.

6. The middle ear will be examined for anatomical variations, such as obstructing mucosal folds over the round window.

7. A micro syringe will then be used to withdraw 20 micro litres of perilymph from the round window

8. Serum for drug levels will be taken at …

9. The rest of the procedure will then proceed as planned

5.3 MEASUREMENT TOOLS

The perilymph and serum samples will be sent for analysis using the liquid chromatography - tandem mass spectrometry detection method to ascertain the drug concentration

5.4 SAFETY

1. Patients who do not meet the inclusion/exclusion criteria will not be enrolled
2. A WHO surgical safety checklist will be performed to ensure that all members of the theatre team are aware of the planned elective procedure including the possible side effects of the drug-poloxomer gel
3. As part of the routine care of patients undergoing general anaesthesia for resection of vestibular schwannoma there will be constant monitoring of the following

    i. Heart rate and oxygen saturation

    ii. Blood pressure through non-invasive blood pressure

    iii. Cardiac status (Routine 3 lead ECG)

5.4.1 Review and Termination of study

1. If an intra-operative adverse event occurs, that could be attributed to the adenosine ligand (eg. cardiac arrhythmias) an independent committee will be conducted lead by the hospital. If it is deemed that the ligand is the likely cause of the adverse event then the dosing will be reviewed and potential review of further steps surrounding the adverse event that could be implemented to prevent this happening again.
2. If there is no way to further minimise the risk of adverse events to patients the study will be terminated as guided by an independent panel.

6. DATA COLLECTION

The following data will be collected, as completely as possible, for every individual in addition to the liquid chromatography - tandem mass spectrometry detection of perilymph ligand levels

- Demographic data.
    - Age, sex and ethnicity.
- Biochemistry results
    - Preoperative — Creatinine, Liver function tests
    - Intra-operative - Serum levels of adenosine ligands

- Intra-operative adverse events

6. Statistical Methods

6.1. Determination of Sample Size

This exploratory study will be used to assess data variability and allow power calculations in future studies.

6.2. Statistical and Analytical Plans

In general, data will be summarized using descriptive statistics (mean, median, standard deviation, minimum and maximum) or frequency counts and percentages, as appropriate to the type of data.

6.3. Handling of Missing Data

There will be no imputation for missing data.

7. Study Documentation

7.1. Protocol Amendments

No amendments to the protocol may be implemented without prior approval from the Research Office and appropriate Ethics Committee (EC), except when the change involves only logistical or administrative aspects of the study.

7.2. Protocol Deviations

Serious protocol breaches must be reported according to the local reporting requirements. The nature and reasons for the protocol deviations will be recorded.

8. Ethics

8.1. Ethics Committee and Regulatory Approval

The protocol will be submitted for approval to the University of Otago Human Ethics Committee (Health). Prior to study initiation, the investigator will provide the Research Office with a copy of the written Ethics Committee (EC) approval of the protocol. This approval letter will identify the study protocol by protocol title, version, and date. If approval is suspended or terminated by the EC, the investigator will notify the Research Office immediately. It is the responsibility of the investigator to report study progress to the EC as required or at intervals not greater than one year.

8.2. Ethical Conduct of the Study

The investigator and all parties involved in this study will conduct the study in adherence to the ethical principles based on the Declaration of Helsinki, ICH GCP guidelines, and applicable national and local laws and regulatory requirements.

9. Data Management

9.1. Data Collection

Data will be collected from the following source:

- Health Connect South.

Data will be collected by the named investigators on the front of this application. Collection of data will be limited to information necessary for the specified purposes of the study.

9.2. Use Of Data

Collected data will be used to answer the research questions described in the protocol.

9.3. Forms Of Data

9.3.1. Identifiable Data

Data will be collected in identifiable form. Identifiable data may be accessed by Michael Bergin, and (if required) the approving IEC.

Data will be collected on a password protected Excel spreadsheets, on a password-protected personal computer. Identifiable data will be retained for at least 10 years.

3.2 De-Identified Data

Identifiable data will be converted to a de-identified form by Michael Bergin prior to analysis.

De-identified data will carry a unique subject code only. Michael Bergin will retain a log linking subject code with NHI identifier. This log will not be made available to other investigators.

De-identified data will be stored on a password protected Excel spreadsheets, on a password-protected personal computer.

De-identified data may be accessed by study investigators, *if indicated* the consulting statistician, and (if required) the approving EC.

The de-identified dataset (or parts there-of) may be presented in study results, including but not limited to peer-reviewed publications and/or scientific meetings. The de-identified dataset may be made available to other approved researchers on request.

The de-identified dataset will be retained for at least 10 years.

9.4. Consent For Data Collection And Use

A waiver of consent will be requested from the EC approving the study (see Section 8.2.1).

9.5. Māori Consultation

Personal and health information is a tāonga and will be treated accordingly.

Formal Māori consultation will be completed as part of the Locality approval process. Any recommendations for additional measures to improve Māori rights and interests in relation to data will be acted upon.

9.6. Breach Of Privacy / Confidentiality

In the event that confidentiality is breached during the study, the following steps will be taken:

- Where possible, the recipient of the data will be contacted and asked to destroy any electronic or hard copies of the disclosed data.
- Affected individuals will be informed of the breach, if indicated, and provided with support as required.
- A review will be conducted to ascertain factors contributing to the breach, and any corrective action required to prevent future breaches.
- The approving EC will be informed.

It is the Investigator's responsibility to comply with legal and regulatory requirements regarding the privacy and confidentiality of subject data.

10.FINANCING AND INSURANCE

A grant application has been submitted to the Eisdell Moore Centre for research totalling $25,000 to be used for the purchase of the gel and adenosine ligands.

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APPENDIX 1

Intra-tympanic adenosine receptor ligands information sheet for patients

Recent studies have demonstrated the ability of certain drugs (Istradefyline and Regadenoson) to improve hearing levels after damage by noise, chemotherapeutic agents and potentially surgery. These drugs are already in clinical use for different indications. The next steps are to prove the ability to deliver these drugs to the ear and establish their pharmacokinetics (drugs action) in the middle ear. We specifically want to establish how much of the drug passes into the inner ear where it will cause the desired effect. It is normally surround by thick bone and is difficult to access

Clinician delete as appropriate

- As part of the operation for a vestibular schwannoma the inner ear is opened, thus allowing us to easily sample the inner ear without causing any further damage.
- As part of Cochlear Implantation the inner ear is opened, thus allowing us to easily sample the inner ear without causing any further damage.

At the commencement of the operation (after you are asleep) a needle will be used to deliver the drug through the ear drum. We will then proceed with the operation. Prior to us opening the inner ear a very small amount of inner ear fluid will be removed and sent for testing.

Throughout the operation your heart rate, blood pressure and heart function will be constantly monitored as part of the routine general anaesthetic safety.

Blood tests will be taken to measure the levels of the drugs during and immediately after the operation

Appendix 2

Intra-tympanic adenosine receptor ligands consent for patients

I, ……., agree to participate in this study. The specific risks associated with this study have been discussed with me. I have had the opportunity to ask questions.

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(Patient's Signature)

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(Doctor's signature)