Study Protocol

Background

Low birth weight premature neonates are at risk of retinopathy of prematurity (ROP). ROP is the leading cause of childhood blindness in Aotearoa, and if this medical condition is left untreated, it can develop into permanent blindness. Neonates born less than 30 weeks gestational age or a birth weight less than 1250g meet the criteria for routine ROP screening[1]. Routine screening by an Ophthalmologist can detect early signs of retina detachment, allowing for timely treatment.

Phenylephrine and cyclopentolate are medicines that are commonly used to dilate the pupil for retinal viewing. These medicines have been known to cause significant harm to the premature infant. Published adverse reports have included unwanted effects on the central nervous, cardiovascular, gastrointestinal, respiratory and vascular system, with the most significant adverse effect being a sentinel event [2-11].

From the limited published information, it is known that sufficient pupil dilation, above 5mm, occurs at small doses [12]. It is counter intuitive to be inflicting potential harm by using medicines at doses far exceeding that required for adequate pupil dilation effects, especially with well known harm associated with these medicines.

There are anecdotal reports amongst colleagues, and suggestions within the published literature, that infants with dark irides may require larger doses of mydriatics due to the hypothesised increased absorption of medication into dark irides.[13, 14] One of the objectives of this study is to consider if eye pigmentation is a factor to consider with regards to medication effects and resulting pupil dilation.

The purpose of the randomised clinical intervention per-protocol pilot study, is to gather information to enable for the best possible design of a larger trial. It is anticipated that irrelevant data collection will be eliminated from the larger trial. All of these factors will contribute to improving the sample size calculation. The testing of the protocol, case report form and other documentation will allow for reflection and modification if required for the larger study. Any areas of potential bias will be noted and used to help develop the large study.

Objectives

Primary objective

- To identify whether phenylephrine 1% (140ug) or 0.5% (70ug) and cyclopentolate 0.2% (28ug) or 0.1% (14ug) in a 7 µL volume will achieve adequate pupil dilation (>5 mm) for retinal examination in premature neonates.

Secondary objectives

- To describe the time-course of physiological markers of systemic absorption following eye drop administration (blood pressure, heart rate, feed intolerance).
- To determine if eye pigmentation (light or dark) is a potential covariate for phenylephrine and cyclopentolate induced pupil dilation.
- To develop an eye colour chart to determine light iris vs dark iris.

Methodology

Participants

Neonates admitted to the Dunedin Hospital NICU, undergoing routine ROP screening or those neonates whom require pupil dilation for red reflex testing.

Participant demographic information that will be recorded: gender, ethnicity (according to neonates whānau/parent(s)/caregiver(s)), dark or light iris colour, gestational age, weight, level of respiratory support (if any) and grade of retinopathy of prematurity.

Inclusion criteria

Refer to appendix 4 for further details.

- All neonates that meet the Dunedin Hospital, Neonatal Intensive Care Unit (NICU) ROP Screening criteria.
- All infants who require pupil dilation for red reflex testing.

Exclusion criteria

- Any neonate with ROP greater than stage 2.
- Any neonate with an ocular medical condition.
- If the staff member who administered the eye drop was unsure if the microdrop reached the eye, then the neonate will be excluded from the trial, in accordance with the per-protocol study design.
- Any neonate whom phenylephrine and/or cyclopentolate is contraindicated.

Treatment

Participants will be randomised to receive either a combination of;

1. One combination microdrop (7 µL) of both phenylephrine 1% and cyclopentolate 0.2% to both eyes.
2. One combination microdrop (7 µL) of both phenylephrine 0.5% and cyclopentolate 0.1% to both eyes.

Proprietary products used during study:

- Phenylephrine (Minims® phenylephrine hydrochloride 10%, manufactured by Bausch & Lomb) 0.5mL eye drops.
- Cyclopentolate (Minims® cyclopentolate hydrochloride BP 1%, manufactured by Bausch & Lomb) 0.5mL eye drops
- Hypromellose 0.5% (Methopt® manufactured by Aspen Pharma)

To achieve the desired concentrations, both minims will be diluted as follows:

<u>Phenylephrine 1% & cyclopentolate 0.2% combination</u>

1. Prepare 0.7 mL of hypromellose 0.5% (Methopt®) in a 3mL syringe
2. Prepare 0.1 mL of phenylephrine 10% (Minims® phenylephrine hydrochloride BP) in a 1mL syringe
3. Using a tip connector, transfer 0.1 mL of phenylephrine to the 3mL syringe (that contains the hypromellose)

4. Prepare 0.2 mL of cyclopentolate 1% (Minims® cyclopentolate hydrochloride BP) in a 1mL syringe
5. Using a tip connector, transfer 0.2 mL of cyclopentolate to the 3 mL syringe (that contains the hypromellose and phenylephrine)
6. Add a small volume of air into the syringe and gently invert the syringe to mix

<u>Phenylephrine 0.5% and cyclopentolate 0.1% combination</u>

1. Prepare 1.4 mL of hypromellose 0.5% (Methopt®) in a 3mL syringe
2. Prepare 0.1 mL of phenylephrine 10% (Minims® phenylephrine hydrochloride BP) in a 1mL syringe
3. Using a tip connector, transfer 0.1 mL of phenylephrine to the 3 mL syringe (that contains the hypromellose)
4. Prepare 0.2 mL of cyclopentolate 1% (Minims® cyclopentolate hydrochloride BP) in a 1mL syringe
5. Using a tip connector, transfer 0.2 mL of cyclopentolate to the 3 mL syringe (that contains the hypromellose and phenylephrine)
6. Add a small volume of air into the syringe and gently invert the syringe to mix

The table below outlines the timing of medication administration for the pilot study:

Time (min)	**Standard treatment**	**Total dose of medication (rounded)**	**Lower dose treatment**	**Total dose of medication (rounded)**
0	Take baseline pupil diameter measurement of both eyes		Take baseline pupil diameter measurement of both eyes	
5	One microdrop (7 µL) phenylephrine 1% and cyclopentolate 0.2%	140 µg/28 µg	One microdrop (7 µL) phenylephrine 0.5% and cyclopentolate 0.1%	70 µg/14 µg
45	Measure pupil dilation of both eyes. If 5 mm or above, administer local anaesthetic and sucrose (as per protocol), and proceed with ROP screen. If below 5 mm, administer second dose as listed below:		Measure pupil dilation of both eyes. If 5 mm or above, administer local anaesthetic and sucrose (as per protocol), and proceed with ROP screen. If below 5 mm, administer second dose as listed below:	
50	One microdrop (7uL) phenylephrine 1% and cyclopentolate 0.2%	280 µg/56 µg	One microdrop (7 µL) phenylephrine 0.5% and cyclopentolate 0.1%	140 µg/28 µg
90	Measure pupil dilation of both eyes. If 5 mm or above, administer local anaesthetic and sucrose (as per protocol), and proceed with ROP screen. If below 5 mm, administer third dose as listed below:		Measure pupil dilation of both eyes. If 5 mm or above, administer local anaesthetic and sucrose (as per protocol), and proceed with ROP screen. If below 5 mm, test has failed and administer	

			third dose as listed below:	
95	One microdrop (7uL) phenylephrine 1% and cyclopentolate 0.2%	420 µg/84 µg	One microdrop (7 µL) phenylephrine 1% and cyclopentolate 0.2%	280 µg/56 µg
120	Ophthalmologist procedure: measure pupil dilation of both eyes and administer local anaesthetic and sucrose (as per protocol).		Ophthalmologist procedure: measure pupil dilation of both eyes and administer local anaesthetic and sucrose (as per protocol).	

Randomisation

The open study will have the participants randomised according to block randomisation, in opaque envelopes of two blocks of eight.

Pupil diameter

To be carried out by two people, and will either be the student researcher and a NICU staff member(s), using both a Colvard Pupillometer and a RetCam. The purpose of two independent measurements is to help validate pupil diameter readings and allow for observer variability.

Primary efficacy outcome measurement

- Pupil measurement will be taken from both eyes at 45 minute.

Secondary efficacy outcome measurement

- Pupil measurements will be taken from both eyes at baseline, 20 minute and 90 minute and 120 minute.
- Ease of ROP screen for Ophthalmologist (easy vs difficult)

Ambient Light

At the time of the pupil measurement, the local illuminance will be measured using a Lux Meter. Measurement of ambient light will allow for calculation of variances between participants baseline pupil results.

Physiological measurements

Baseline blood pressure will be taken. Subsequent measurements will be taken immediately prior to pupilometer measurements then approximately 6hrly post mydriatic dosing for 24 hours. Phillips monitor will be used to take a manual blood pressure.

Heart rate will be continuously measured for 24 hours prior and 24 hours post eye drop installation. Phillips monitor will be used to record heart rate.

Temperature will be recorded at baseline, 1hr (range 45min to 90 min) post eye drop administration. Temperature will be measured from the axilla, using Welsh/Allyn Sure Temperature Plus digital thermometer.

Feed intolerance will be reviewed by retrospectively reviewing feed volumes and spills, on either the category 4 or 5 observation chart, for 24 hours prior and 24 hours post eye drop installation. Medical notes will be reviewed retrospectively for any documentation of Nectrotising Enterocolitis for 7 days post mydriatic eye drop installation.

Treatment emergent adverse events (TEAE)

- Serious treatment related TEAE – discontinuation of treatment due to adverse effects
- Treatment related AE – prolonged crying, vomiting

Eye colour chart

All neonates will have a photo taken of their eye with the RetCam. Photos will be collated to develop a reference to determine whether an infant has light or dark iris.

Statistical Analysis

Will be carried out by Jill Hazard, Statistician and Lisa Kremer, Masters student. Refer to appendix 1 for further detail regarding statistical analysis.

An assumption is made that data is missing completely at random. Missing data will not be imputed. Data will remain missing and will be reported.

Test

Data from test will be recorded on a case report form and will be de-identified. See Appendix 7.

A successful ROP screen, and therefore sufficient pupil dilation, will be deemed by the visiting Ophthalmologist.

Ethical issues

Consent will be obtained from whānau (preferably not an individual but from a collective of 2 or more) by the NICU Research Nurse or by any other trained NICU staff member who is not the primary caregiver or main Paediatrician or Neonatologist caring for the neonate. Refer to appendix 5 and 6 for further details.

Consultation with Māori

Consultation with the Ngāi Tahu Research Committee within the University of Otago and with Hine Forsyth has taken place and recommendations actioned. Refer to appendix 2 and 3.

Dissemination of Findings

- Whānau, parent(s) or caregiver(s) will be offered to be send findings from the trial.
- Findings will be sent to Mark Brunton, Research Manager, The Office of Māori Development, University of Otago, as recommended by the Ngāi Tahu Research Committee.
- Findings will be sent to Hine Forsyth, to be distributed to Ngāi Tahu if any information is found that is of relevance to Māori.
- Findings, if accepted, will be published in neonatal/paediatric relevant journals.
  Findings, if accepted, will be presented at neonatal/paediatric relevant conferences.

References

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