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Systemic Absorption of Cyclopentolate and Adverse Events After Retinopathy of Prematurity Exams

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Abstract

Purpose—Preterm infants undergoing Retinopathy of Prematurity Eye Exams (ROPEE) may experience adverse events, possibly from systemic absorption of cyclopentolate. The purpose of this study was to analyze the association between adverse events and drug levels found in neonates undergoing ROPEE.

Materials and Methods—25 infants were randomized into two groups during routine ROP screening: 5 infants for blood collection before mydriatic drops and 20 for blood collection 1 h after eye drops. Blood was collected onto dried blood spot cards, extracted, and analyzed for cyclopentolate and phenylephrine using liquid chromatography and mass spectrometry. Relationships between drug levels and adverse events were assessed.

Results—Cyclopentolate (range 6–53 ng/ml) was observed in 15 of 18 infants, while phenylephrine was not detected. Levels of cyclopentolate were significantly higher in infants who were on oxygen ($p = 0.01$). There was a significant association between cyclopentolate levels and gastric residuals in tube-fed infants not receiving oxygen ($p = 0.01$).

Conclusions—Cyclopentolate levels varied among preterm infants after ROPEE. Cyclopentolate was positively associated with increased gastric residuals. Underlying medical conditions requiring oxygen administration may affect absorption and metabolism of cyclopentolate. There is a need to predict infants at risk for high blood levels of cyclopentolate in order to prevent or treat adverse events after ROPEE.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Keywords

Adverse events; cyclopentolate; preterm infant; retinopathy of prematurity; screening exams

Introduction

Preterm births are a major public health problem in the United States where each year about 58,000 preterm infants are born weighing <1500 g.¹ These infants are at increased risk for medical and developmental complications at birth. One common complication of preterm birth is retinopathy of prematurity (ROP), a disease of the developing blood vessels on the retina that may lead to vision impairment or blindness.² The American Academy of Pediatrics (AAP) has published guidelines for ROP screening examinations that start at 4–6 weeks of age, focusing on infants born at less than 30 weeks gestational age or weighing less than 1500 g.^{3,4} These examinations require administration of mydriatic ophthalmic drops to dilate the pupils and sometimes involve physical manipulation of the eye. Ocular administration of cyclopentolate alone or in combination with phenylephrine is the preferred drug used to induce pupil dilation in infants.⁴ Cyclomydril®, a mydriatic containing both cyclopentolate (0.2%) and phenylephrine (1%), was used in the present study.

While the data have shown that cyclopentolate does not cause adverse events severe enough to prevent the eye exam,⁴ some infants experience adverse physiological changes after ROPEE that may be attributable to cyclopentolate (0.2%), a muscarinic antagonist,⁵ or phenylephrine (1%), an adrenergic agonist.^{6–8} These agents are routinely administered 40 min to 2 h prior to examination.^{9–15} One pilot study tracked several physiologic changes in 50 preterm infants undergoing their first ROPEE and found a significant increase in apnea 24–48 h after the ROPEE.¹⁶ Within the scope of cyclopentolate pharmacological response, it is reasonable that cyclopentolate might cause adverse events if it is systemically absorbed. There have been a few reports and case studies showing the extent of cyclopentolate absorption following administration to the eye.

Preterm infants may be especially susceptible to the systemic effects of cyclopentolate because the drug is administered as a unit dose and not precisely tailored for this population. Thus, infants would receive a relatively higher exposure to the drug than adults since their circulating blood volume is much less than that of an adult. In addition, infants' drug metabolism and excretion systems are immature, prolonging the half-life of many drugs.^{17,18} Previous reports of the extent of cyclopentolate absorption following ocular administration have shown high variability in the time to maximum concentration ($t_{\max}$) and in the maximum concentration ($C_{\max}$). Lahdes reported an average $t_{\max}$ of 30 min and elimination half time of 111 min in adult subjects ($n = 8$).¹⁹ Interestingly, a second cyclopentolate peak was observed in some of the subjects in that study. This second peak was presumed to come from oral absorption of cyclopentolate via the nasolacrimal duct. While it is possible that the expression of functional cholinesterase may play a role in poor clearance of cyclopentolate, the evidence from case studies reported above strongly suggest a genetic predisposition to poor enzymatic function.²⁰

Despite noting systemic pharmacodynamic effects in neonates following cyclopentolate and phenylephrine administration, nothing is known about the extent of systemic absorption of cyclopentolate and phenylephrine following ophthalmic administration to preterm infants. Specifically, both the amount of systemic absorption and the effects of these drugs on preterm neonates are unknown, likely because of the blood volume required to quantitate these levels. Herein, a novel method to measure cyclopentolate and phenylephrine levels in preterm infants using small volumes of blood was developed to analyze these levels and assess the relationship of cyclopentolate to adverse events after ROPEE.

Materials and methods

Study subjects

The study was approved by the University of Arkansas for Medical Sciences Institutional Review Board and the director of the Neonatal Intensive Care Unit (NICU). Parents of eligible infants were contacted and research staff met with parents face-to-face to discuss the study, allow time for questions, and obtain written consent before infants were enrolled in the study. Any infant who was scheduled for ROPEE was eligible for the study. The NICU used guidelines slightly modified from the AAP Guidelines.³ Infants were scheduled for ROPEE if they were born at less than 32 weeks (NICU policy) and/or weighed less than 1500 g. They ranged in age from 4 to 6 weeks. Many of these infants received a series of ROPEEs, but for consistency and to reduce extraneous variables, the study was carried out on the first eye exam only. Twenty-eight infants were enrolled, with three discharged from the NICU prior to their first ROPEE. Twenty infants were randomized to a group whose blood was collected 1 h (± 15 min) after cyclomydril administration, and five randomized to a control group whose blood was collected before cyclomydril administration.

Data collection phase 1

The same pediatric ophthalmologist performed all ROPEE on all eligible infants enrolled in the study. Pupils were dilated approximately 2 h before the ROP eye examination by a registered nurse who had special training and experience with eye examinations. One drop of cyclomydril was instilled into each eye every 5 min for a total of three doses. The total dose administered was 600 μ g cyclopentolate (0.2%) and 3 mg phenylephrine (1%). During instillation of eye drops, cardiorespiratory monitors were in use with alarms on, and infants received the same care as other infants in the unit undergoing ROPEE.

Infants in the NICU undergo routine metabolic laboratory work on a weekly basis. With permission of the NICU medical staff, this routine weekly laboratory work was timed to be collected at the same time that the two drops of blood were collected for this study. Registered nurses in the NICU who routinely collect blood carried out this procedure by placing a small 24-gauge needle into a superficial vein, allowing the required amount of blood to drip into the specimen tube. After the blood was collected, two drops were placed onto a FTA DMPK-C card (GE Health Life Sciences, Pittsburg, PA, USA). The cards were allowed to air dry for approximately 15 min, transferred to the bioanalytical laboratory, and stored at -80°C until analyzed by LC-MS/MS. The sample collection cards were coded by number only and did not include any patient identifying information.

Prior to extraction of blood, cards were allowed to warm to room temperature in a desiccator for 30 min and then three 3.0 mm spots were removed from each using a Harris Unicore punch (GE Health). These punches were combined and added to 100 μ l of solution containing 80% acetonitrile and 20% water. This was sonicated for 30 s and centrifuged at 10 000 *ref* for 5 min. The supernatant was then filtered through 0.45 μ m nylon centrifugal filters (EMD Millipore).

LC-MS/MS analysis

Extracted samples were analyzed on an Acquity uHPLC system coupled to a Premier triple quadrupole mass spectrometer (Waters Corporation, Milford, MA, USA). Prior to detection by tandem mass spectrometry, analytes were separated on a 100 $\times$ 2.1 mm phenyl hexyl (3 μ m) column (Thermo Scientific, Waltham, MA, USA) using a linear acetonitrile gradient with a total run time of 8 min. The mobile phase was modified with 0.1% formic acid and the flow rate was 0.6 ml/min. Positive ions were generated using electrospray ionization at a cone voltage optimized for each analyte. Product ions were generated by collision-induced dissociation using argon at a pressure of 2×10^{-3} Torr and an energy optimized for each analyte. Phenylephrine and cyclopentolate ($[M+H]^+$) were detected at m/z 168.3 $\rightarrow$ 150 and 292 $\rightarrow$ 274.1, respectively. Phenylephrine- d_3 and caffeine- d_9 served as internal standards. The lower limit of quantitation was defined as the concentration resulting in a signal-to-noise of 10. The lower limit of quantitation for cyclopentolate and phenylephrine was 5 ng/ml and 10 ng/ml.

Data collection phase 2

Medical records of infants who participated in the first phase of this study were reopened with IRB approval, and adverse events experienced the day before and the day of ROPEE were counted. Monitored adverse events included bradycardia (defined as a heart rate less than 80 beats per minute for 10 s); oxygen desaturation (defined as an oxygen saturation less than 80% for greater than 10 s); need to increase supplemental oxygen; apnea (defined as a pause in respiration greater than 10 s or if associated with systemic signs or color change); increased gastric residuals, indicating feeding intolerance, and other gastrointestinal complications (including vomiting); and any unusual neurological events (including seizure activity or unusual sleepiness). Gestational age was based on best obstetrical estimate. Data collected during the chart review were coded by study number only and no identifying information was recorded. This was a blinded review, and cyclopentolate levels were not compared with adverse events until the review was complete.

Results

Phase 1

Drug levels were determined in 18 out of 20 patients randomized to the post-administration blood draw group. Data from two subjects are not reported here, since these samples were extracted and analyzed during the development phase of the analytical method and prior to final validation of the method. Quantifiable blood levels of cyclopentolate were observed in 15 out of these 18 infants. There were 7 male and 11 female, 14 Caucasian, 3 African American, and 1 Asian. The mean gestational age was 28.5 weeks (SD 2.8) and the mean

birth weight was 1148 (SD 523). The highest blood concentration of cyclopentolate was 53 ng/ml and the median cyclopentolate level was 17 ng/ml (range = 6–53 ng/ml). In three of the infants, the level of cyclopentolate was below the lower limit of quantitation (<5 ng/ml). Phenylephrine blood levels were below the lower limit of detection (LOD), and similarly cyclopentolate and phenylephrine blood concentrations were below the LOD in samples taken before administration of cyclomydril ($n = 5$).

Phase 2

Ranges of cyclopentolate levels and associated adverse events are shown in Figure 1. Cyclopentolate levels were divided into three groups a priori based on how the data were clustered. It was noted that five out of six infants in the group with the highest cyclopentolate levels (24–53 ng/ml) were infants who were more medically fragile and on respiratory support prior to ROPEE. Respiratory support included nasal cannula airflow, supplementary oxygen, or ventilator support. Cyclopentolate concentrations for infants receiving oxygen therapy at the time of cyclomydril administration were significantly higher than for infants who were not receiving oxygen ($p = 0.01$, Figure 2).

As shown in Figure 1, there was an increased frequency of adverse events in the group with the highest cyclopentolate levels (24–53 ng/ml). However, using multivariable logistic regression to control for patient-on-oxygen, there were no statistically significant associations between cyclopentolate levels and change in bradycardia events, change in desaturation events, unusual neurological events, or gastrointestinal events other than increased gastric residual. Figure 3 presents scatterplots of three adverse outcomes (bradycardia, oxygen desaturation, increased gastric residual) versus cyclopentolate concentration, stratified by patient-on-respiratory-support. The only statistically significant relationship was among infants who were *not* already receiving oxygen. There was a positive, statistically significant relationship between cyclopentolate concentration and change in gastric residual volume after treatment ($p = 0.01$), but not among infants who were already receiving oxygen ($p = 0.47$). Cyclopentolate levels and change in gastric residual levels were both high in the oxygen group. The positive slope in panel B for bradycardia was leveraged by one patient with an increase of 12 bradycardias from pre- to post-ROPEE and was not statistically significant ($p = 0.08$).

Infants receiving oxygen treatment tended to have lower gestational ages, but there was no statistically significant relationship between gestational age and cyclopentolate concentration, whether or not controlling for patient-on-oxygen. Lower birth weights were associated with higher cyclopentolate concentrations ($p = 0.04$), but this relationship was not statistically significant after controlling for patient-on-oxygen in a multivariable linear regression ($p = 0.63$).

Discussion

Our findings are in agreement with several other studies demonstrating increased adverse effects including vomiting, increased gastric residuals, and apnea after ROPEE.^{3,21–23} However, this is the first study we are aware of that has shown the relationship of increased adverse events to specific levels of the anticholinergic drug cyclopentolate. While it has been

known for some time that ROPEE can result in adverse effects,^{21,24} it has not been known whether the pain and stress of the eye exam or the drug itself caused these effects. This study supports a causal relationship of cyclopentolate leading to adverse events.

Levels of cyclopentolate found in this sample of infants (range below the LOD to 53 ng/ml, median 17 ng/ml) were about 10-fold higher than levels reported earlier in children and adults.^{25,26} Cyclopentolate plasma levels in children after administration of one drop of 1% cyclopentolate in each eye for a total dose of 1 mg have ranged from undetectable to 5.8 ng/ml (median 2.9 ng/ml),²⁶ much lower than findings in this study. This is a significant and sobering finding in vulnerable preterm infants and raises questions about how this population is metabolizing the drug and how the drug might be affecting them. While we know that infants may suffer side effects of cyclopentolate, we do not know what blood level of the drug would be considered a toxic level.

Phenylephrine was not measurable in the blood samples, probably due to the poor permeability of phenylephrine ($pK_a = 9.2$) in the acidic environment of lacrimal fluid. While phenylephrine was not detected following cyclomydril administration, this does not mean that phenylephrine was not absorbed; rather it was probably distributed rapidly to peripheral tissues, as in adults.²⁷ The pK_a for cyclopentolate is 8.2, suggesting that more of the cyclopentolate will be in the free base form and thus more membrane soluble than phenylephrine. The $\log P$ -value for cyclopentolate ($\log P = 1$) is also 10-fold higher than that of phenylephrine ($\log P = 0.1$), indicating higher passive diffusion potential for cyclopentolate.

Our study found increased bradycardia and oxygen desaturation events after ROPEE, but the infants who showed the greatest increase in these events and had the highest cyclopentolate levels were also those who were sicker and on some type of respiratory support. After adjusting for patient-onoxxygen, increased bradycardia and desaturation events were not statistically significant. However, this association does indicate the need for close clinical monitoring after ROPEE, especially for those patients on oxygen. Other studies have found that preterm infants experience adverse events such as bradycardia and oxygen desaturation after ROPEE.^{9–15} Preterm infants may be especially susceptible to systemic effects because their blood volume is much less than that of an adult. In addition, drug metabolism and excretion systems are immature, prolonging the half-life of most drugs.^{28,29}

Bradycardia can be caused by a physiological response termed oculocardiac reflex. Oculocardiac reflex is caused by stretching of the ocular muscles pressure on the ocular globe and a subsequent suppression of heart rate (e.g., bradycardia).³⁰ This response is counter to what would be expected from muscarinic antagonist, like cyclopentolate. While cyclopentolate has not been shown to produce this response, Ohashi and co-workers have shown that atropine prevents bradycardia following physical stretching of the ocular muscles in adult subjects.³⁰ The cardiovascular response following systemic adsorption of muscarinic antagonist is complex.³¹ At low doses of atropine (0.1 $\mu\text{g/kg}$ –3 $\mu\text{g/kg}$), adults experienced bradycardia, while at higher doses (10 $\mu\text{g/kg}$ –50 $\mu\text{g/kg}$), tachycardia has been observed.³¹ The heart rate decreasing effect of low-dose atropine has been attributed to blockage of M1 subtypes that normally limit acetylcholine release in the sinus node. At

higher atropine doses, the drug acts on the M2 receptor subtype and causes tachycardia. In order to understand and explain the activity of cyclopentolate, a clear understanding is needed of its selectivity, efficacy, and potency as a muscarinic antagonist.

Five muscarinic receptor subtypes have been identified (M1–M5), and each subtype shows specificity in terms of anatomical location and function.^{32,33} Historically cyclopentolate has been described as a non-specific muscarinic antagonist, but there is no evidence supporting the nonspecificity at muscarinic receptors.

There is a need for health care providers to be aware of possible life-threatening events after mydriatic eye drop administration for ROPEE. Although apnea was only found in one infant in the current study, in a previous study we found significant apnea in infants ($n = 50$) 24–48 h after their ROPEE ($p < 0.04$).¹⁶ This is a concern in preterm infants because decreased cerebral perfusion may result from the bradycardia, hypotension, or decreased oxygen saturation.³⁴ Infants who undergo ROPEE on an outpatient basis or who are discharged on the day of their eye exam may require additional monitoring at home.

The study did find significantly increased gastric residuals and a positive correlation between cyclopentolate levels and gastric residuals among infants who were not on oxygen. The anticholinergic effects of cyclopentolate ophthalmic drops may slow peristalsis and result in abdominal distention, increased gastric residuals, and vomiting.^{35,36} Vomiting was noted in two infants in this study after ROPEE, but the sample was too small to be conclusive. In support of these findings, another study showed that the feeding performance of 50 infants undergoing ROPEE was compared for 24 h before and 24 h after the eye examination. Duodenal motor contractions decreased fourfold after the eye examination, and gastric emptying was significantly delayed.³⁵ Feeding intolerance from the effects of cyclopentolate may adversely affect nutrition and growth, but additional translational research is needed in this area.^{37,38} Of more concern are the potential effects on possible aspiration and apnea. Since many of these examinations are done in an outpatient setting, these effects could lead to cardiopulmonary arrest in a setting ill equipped to handle these events.²¹

It is relevant to note that all infants received the same dose of cyclopentolate, but some infants had no measurable levels of cyclopentolate while others had levels higher than those reported for children and adults.^{25,26} These variations in blood levels are clinically significant and indicate a need to determine possible genetic influences on metabolism of this drug.^{17,39} Genes that influence the metabolism of anticholinergics have been identified, and it is possible that one or more of these candidate genes may contribute to our findings.^{20,32,40–43}

An unexpected finding in this study was that infants who were receiving oxygen with some form of respiratory support had higher levels of cyclopentolate than other infants, even though all infants received the same dose of eye drops. Whether or not the infant was on oxygen was the strongest predictor of cyclopentolate levels. This indicates that this physiologically vulnerable group of infants with chronic lung disease is at risk for metabolizing and clearing cyclopentolate and may need special considerations such as a

lower dose of the drug. The reason for this finding is unknown, and it is likely that oxygen is a marker for other factors such as low birth weight or gestational age. Alternatively, since oxygen is a vasodilator, increased oxygen saturation levels may lead to enhanced absorption, a decrease in clearance, or a decrease in the volume of distribution. Since a full description of the pharmacokinetics of cyclopentolate has not been adequately presented here or previously, additional research is needed to confirm and explain this finding.

The determination of circulating drug levels to determine the association of adverse effects after ROPEE with cyclopentolate and phenylephrine administration was unique to this study. This study was limited by the small sample size, indicating the need for a larger study. Some clinical indicators of adverse events such as bradycardia, oxygen desaturation, vomiting, and unusual sleepiness after ROPEE were too few in number to be statistically significant. An additional limitation is the retrospective chart review that was used to identify adverse events. The frequency count of adverse events was dependent on health care providers charting every bradycardia, oxygen desaturation, and vomiting event, and it is possible that some events were overlooked or not recorded, indicating the need for a prospective study design.

There was variation in how infants absorb or metabolize cyclopentolate after ROPEE. Variability in the disposition of cyclopentolate following ocular administration has been observed in adults as well.²⁵ Elevated drug levels are associated with increased adverse events after ROPEE including gastric residuals and possible cardiopulmonary events. More research is needed to analyze infant factors such as the level of illness to determine why some infants absorb and metabolize cyclopentolate differently than others.^{17,39} Approaches to decrease or predict the disposition of this drug in vulnerable sub-populations of preterm infants are urgently needed to prevent the adverse consequences of ROPEE.

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Figure 1.
Adverse events the day before ROPEE compared to the day of ROPEE, grouped by cyclopentolate concentration ranges.

Figure 2.

Cyclopentolate concentrations by patient-on-respiratory-support. Boxplot is overlaid.

Cyclopentolate concentrations for patients on respiratory support are significantly higher than those not ($p = 0.01$, two-sample t -test).

Figure 3. Scatterplots of three adverse outcomes vs. cyclopentolate concentration, stratified by patient-on-respiratory-support.