

Evaluating the Potentiating Effect of Amethocaine on Tropicamide-Induced Mydriasis in Darkly Pigmented Irides, Using Infrared Pupillometry

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ABSTRACT

PURPOSE: To determine whether prior instillation of amethocaine would increase the rate and magnitude of tropicamide-induced pupillary dilatation in darkly pigmented irides.

METHOD: A total of 50 healthy Africans aged 18–40 years, with darkly pigmented irides, received a drop of amethocaine in one eye and a drop of placebo in the contralateral eye, followed by two drops of tropicamide in both eyes. Serial pupil diameters were measured using a monocular infrared video pupillometer. Rate of pupillary dilatation was compared in both eyes. Survival statistics were calculated for the time taken to reach a clinically effective dilatation, clinically effective diameter (CED) of 6.0 mm.

RESULTS: Mean difference in pupil diameters between amethocaine- and placebo-treated eyes was 0.30 ± 0.09 mm ($P < 0.002$). In all, 62% of amethocaine-treated eyes and only 46% of placebo-treated eyes reached the CED.

CONCLUSION: We observed a small but statistically significant potentiating effect of a drop of amethocaine on tropicamide-induced dilatation within 20 minutes.

KEYWORDS: amethocaine, tropicamide, tetracaine, potentiating effect, pupil, dilatation

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Introduction

Iris color influences the rate and degree of pupillary response to dilating agents. Darkly pigmented irides do not dilate as fast or as well as light-colored irides. However, there have been studies suggesting that prior instillation of an anesthetic like amethocaine may enhance the adrenergic iris dilator muscle response to a mydriatic-like tropicamide, by increasing its corneal penetration and hence its bioavailability.^{1,2} Achieving adequate mydriasis has diagnostic and therapeutic implications; for instance, the visualization of the retinal periphery for the staging or diagnosis of retinal disorders such as retinopathy of prematurity³ retinoblastomas,⁴ or the laser treatment of retinal tumors as indicated in transpupillary thermotherapy⁵ and for

surgical procedures.⁶ While higher concentrations or synergistic combinations of mydriatics may be used to achieve greater mydriasis,⁷ it is reported that simply applying a topical anesthetic before the instillation of a mydriatic not only increases the comfort of the patient but also increases the rate and degree of mydriasis achieved.^{7,8} Mordi et al. documented a clear potentiating effect of proparacaine on tropicamide-induced mydriasis in blue—green and hazel—brown irides.⁹ However, while the blue—green pupils remained at 90% of maximal dilatation for a longer duration, the duration was not prolonged in brown—hazel eyes. This demonstrates that iris color affects the response of the pupil to mydriatics, being less responsive as the iris color darkens. On the other hand, Ghose reported a significant increase

of 3.62 ± 0.75 mm in pupil size in dark irides pre-treated with lignocaine 4%, when dilated with tropicamide drops, suggesting that pre-instillation of an anesthetic could indeed potentiate tropicamide-induced dilatation in darkly pigmented irides. This controversy therefore prompted this present study to determine and quantify the potentiating effect of a single drop of amethocaine, a topical anesthetic, on the mydriasis, produced by tropicamide, a commonly used dilating agent, in healthy young African adults, with darkly pigmented irides.

Materials

Infrared video pupillometer. An infrared video monocular pupillometer was utilized in this study. The camera module was constructed from a 1/4" monochrome CCD camera board, a specialized constant-magnification lens, and a LED flex printed circuit board populated with two infrared LEDs for iris illumination. The pupillometer was interfaced directly to a laptop PC via an IEEE 1394a port. The software used for control and acquisition of pupil data was a special investigational version of the software (Fig. 1) provided commercially by NeuroOptics™ (Irvine, CA, USA, <http://www.neurooptics.com>) desktop pupillometer systems. The spatial calibration of the system was determined to be accurate within ± 0.03 mm.

Masked eye-drop preparations containing placebo or amethocaine. Four color-coded groups of identical eye-drop pairs labeled A and B were used. Amethocaine and placebo (normal saline) were packaged in pairs of color-coded bottles that were similar in every respect except for their contents. The contents of bottles A and B were randomized to contain amethocaine or the placebo by a clinician who was not involved in data collection or analysis and who kept the information for masking until or after data collection was completed.

Methods

Setting. The study was performed in the Department of Ophthalmology, outside the regular clinic setting to ensure a controlled environment with minimal distraction and consistent ambient lighting (approximately 100 lx) conditions. Written informed consent was obtained from each participant according to the institutional bioethics-approved protocol. The study was performed with strict adherence to the declaration of Helsinki and conducted with the approval of the University College Hospital, Institutional Review Board.

Healthy young adults between the ages of 18 and 40 years, who had not used any topical eye medication in the preceding two months, were included in the study. All subjects had darkly pigmented irides of grades IV and V as categorized by Seddon et al. and shown in Figure 2.¹⁰ Subjects with a history of diabetes, hypertension, eye injury, eye surgery, eye medications, or any known allergy to either amethocaine or tropicamide were excluded. Screening for diabetes and hypertension was done using blood pressure measurement and urinalysis, respectively. Other exclusion criteria were obtained by self-reporting on a standardized questionnaire.

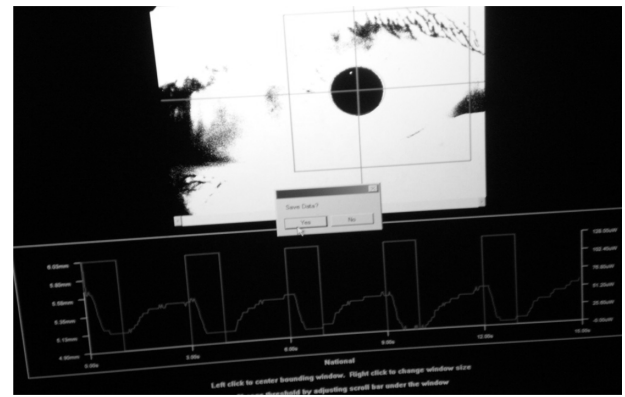


Figure 1. Pupil measurement with software in LED infrared video pupillometer.

Study design. Each subject served as his/her own control. When one eye received the placebo and the tropicamide, the contralateral eye received amethocaine and tropicamide.

Randomization. Each subject was allocated to a color-coded group by simple ballot. Each subject was presented with four identical tokens to choose from, each of which had a hidden code written on it. Once the subject selected a token, the hidden code was checked which indicated the individual's assignment into one of the four coded groups.

Undilated pupil measurement. The undilated pupil diameter in each eye was measured with the infrared pupillometer and recorded at the start of the experiment.

Instillation of drops. The process of eye-drop instillation was standardized to ensure maximal absorption as described by Fraunfelder.¹¹ Eye drops were instilled by the investigator, into the inferior cul-de-sac of each eye with the subject looking up (at the ceiling). Light pressure was applied over the nasolacrimal duct to reduce tear drainage.

Dilatation process. The dilatation process described by Thompson¹² was used to obtain maximal dilatation. Drops A + C were always instilled in the right eye while the left eye received drops B + C. However, the contents of A and B were randomized by simple ballot, and both the investigator and subjects remained blinded throughout the experiments. Unmasking was only done at the conclusion of the study to enable analysis of the results.

Measurement of rate and magnitude of pupillary dilatation. Following instillation of the second and final dilating drop at five minutes, pupil diameters were measured and recorded for each eye, every 10 minutes, for one hour. At one hour, reversal was initiated with the instillation of 2% of pilocarpine into each eye. Pupil diameters were also measured during reversal at 15-minute intervals for another one hour. The experiment concluded with the final measurement of the pupil diameter at 120 minutes (60 minutes post-instillation of pilocarpine).

Results

There were a total of 50 subjects, comprising 29 males and 21 females. The mean age was 27 ± 5.9 years. A total of 24 subjects

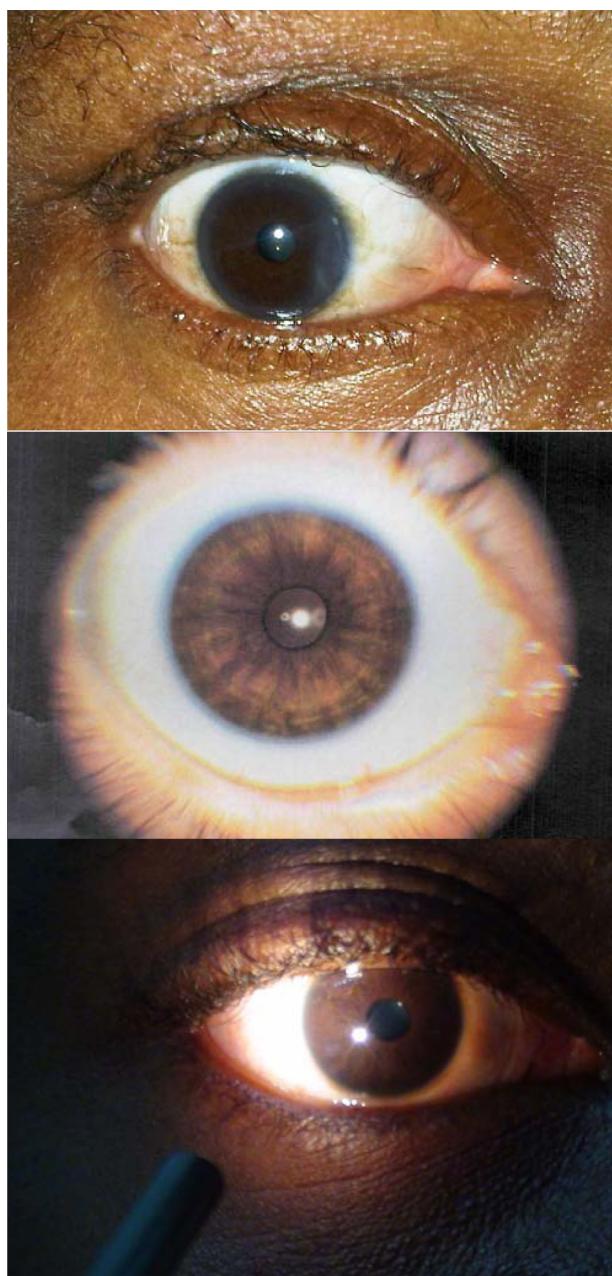


Figure 2. Examples of the darkly pigmented irides in this study, in which the pupil is barely visible without illumination, prompting the use of the infrared pupillometer (middle: Standard D, from Seddon et al., Evaluation of an Iris Color Classification System. Invest Ophthalmol Vis Sci 1990; 31:1592–1598). These irides are darker than standard D (shown) and therefore correspond to color grade V.

received the placebo in their right eye and the amethocaine in the left, whereas 26 subjects received the opposite. Mean undilated pupil diameter was 4.25 ± 0.72 mm in the placebo eyes and 4.15 ± 0.15 mm in the intervention eyes. The mean undilated pupil diameters did not differ significantly between the placebo eyes and drug eyes at the onset ($P = 0.168$). For the purpose of statistical analysis, a pupillary diameter of ≥ 6.0 mm (clinically effective diameter, CED) was taken to be clinically significant.⁸

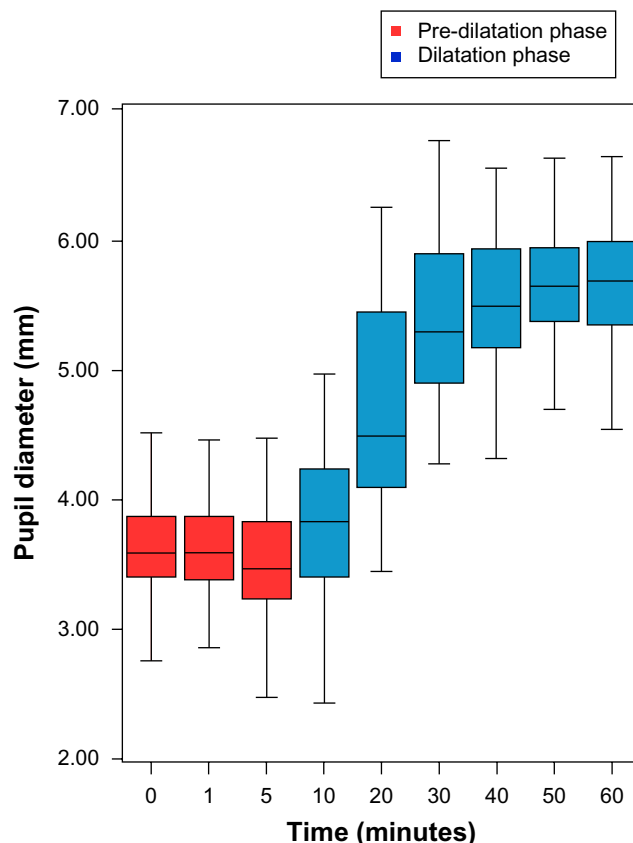


Figure 3. Box-plot showing the pupil diameters in the “Placebo” eyes of 50 subjects at specific intervals during dilatation.

Figure 3 is a box-plot showing the spread of pupil diameters recorded in the control (placebo-treated) eye during the 60-minute dilatation phase. It reflects the unpotentiated mydriatic effect of tropicamide 1%. The median pupil diameter remained relatively unchanged in the first 5 minutes, dipped slightly at 5 minutes, and then increased steadily from 10 to 60 minutes. The most marked change in pupil size occurred between 10 and 20 minutes.

The box-plot in Figure 4 shows a similar pattern of change in the pupil diameters measured during the same intervals in the amethocaine-treated eye. The most marked change also occurred between 10 and 20 minutes.

The exact pupil sizes measured during the period of dilatation are outlined in Table 1.

Table 1 shows the mean pupillary dilatation and the mean difference in pupil size between the amethocaine-treated eye and the placebo-treated eye over time. The pupil diameter, in the amethocaine-treated eye, increased from a mean size of 4.15 ± 0.72 mm at 0 minutes to a mean size of 6.01 ± 0.54 mm at 60 minutes. On the other hand, the mean pupil size in the placebo-treated eye increased from 4.25 ± 0.73 mm at 0 minutes to a mean size of 5.90 ± 0.48 mm at 60 minutes. Significant differences were recorded between the placebo-treated eye and the amethocaine-treated eye five minutes after the dilating drop was instilled, where the highest difference of

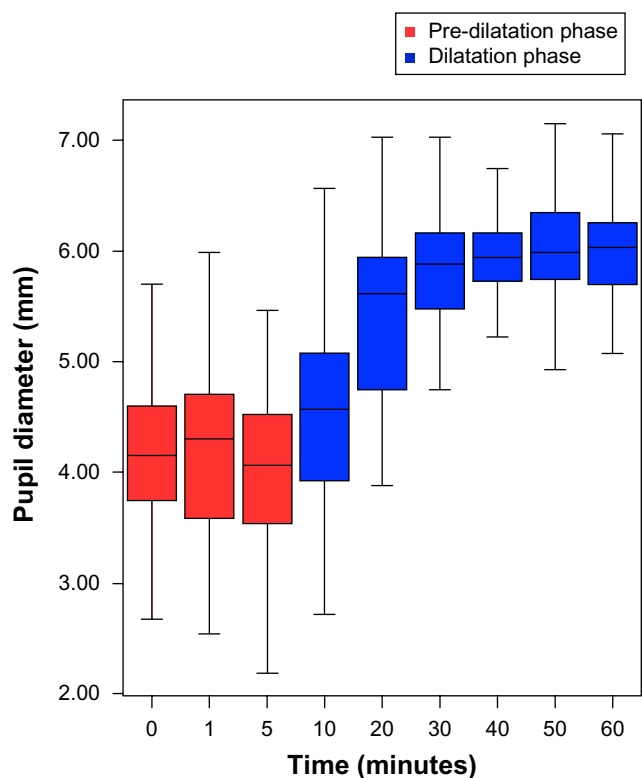


Figure 4. Box-plot showing the pupil diameters in the “Intervention” eyes of 50 subjects at specific intervals during dilatation.

0.3 mm (SD 0.09, $P=0.002$) was observed. This was recorded at the 10–20-minute interval.

Figure 5 is a graphical representation of the change in pupil size (dilatation), measured in millimeters per minute, recorded in the placebo-treated eyes and compared with the amethocaine-treated eyes during the 60-minute dilatation phase. The line graphs show the sum of the mean change in pupillary size with respect to time.

There was a steady increase in the size of the pupils from baseline. While the mean pupil diameter increased by up to 2 mm in the drug eyes, dilatation in the placebo eyes was less than 2 mm. The marked dilatation, which occurred between 10 and 20 minutes in both the placebo-treated and amethocaine-treated eyes, is shown by the wider separation of the two curves during this time interval. Figure 6

analyses the average rate (speed) of dilatation in millimeter per minute. The highest change in average dilatation speed occurred within the first 15 minutes, when the average rate of dilatation increased from a baseline of 0.04 mm/minute in the placebo-treated eye and 0.05 mm/minute in the amethocaine-treated eye at 5 minutes, respectively, to a peak of 0.08 mm/minute in the placebo-treated eye and 0.09 mm/minute in the amethocaine-treated eye at 15 minutes. The average rate of dilatation rapidly slowed down in amethocaine-treated eyes dropping to 0.04 mm/minute at 25 minutes, 0.01 mm/minute at 35 minutes, and becoming negligible thereafter. The speed of dilatation followed a less precipitous initial drop in the placebo-treated eye, dropping to 0.06 mm/minute at 25 minutes and 0.01 mm/minute at 35 minutes, also becoming negligible thereafter. Average pupil dilatation rate was the same in both placebo-treated and amethocaine-treated eyes (0.01–0.005 mm/minute) between 35 and 45 minutes, but dropped further in amethocaine-treated eyes to 0.003 mm/minute while leveling off at 0.01 mm/minute in placebo-treated eyes at the end of the dilatation period.

Table 2 shows that a total of 31 (62%) subjects reached the CED of 6.0 mm, in their amethocaine-treated eye, whereas 19 (38%) did not. On the other hand, only 23 (46%) subjects achieved the CED in their placebo-treated eye whereas 27 (54%) subjects did not. In all, 20 subjects achieved the CED in both their placebo-treated and amethocaine-treated eyes (40%) whereas 16 individuals failed to achieve the CED at all, in either their placebo-treated or amethocaine-treated eye (32%). There were 11 subjects (22%) who achieved the CED in their amethocaine-treated eye but not in the placebo-treated eye and 3 subjects (6%) who achieved the CED in the placebo-treated eye but not in the amethocaine-treated eye.

The McNemar’s odds ratio for achieving the CED of 6.0 mm in the drug eye was 3.67 ($P=0.057$).

The Kaplan—Meier survival curve in Figure 7 illustrates the probability of not reaching the CED in the amethocaine-treated and placebo-treated eyes. The mean estimate of time to reach CED in the amethocaine-treated eyes was 46.4 minutes, whereas in the placebo-treated eyes it was 52.4 minutes. The x -axis shows the time taken to reach the CED of 6.0 mm, in minutes, whereas the y -axis denotes the probability of

Table 1. Mean pupil size and mean difference between placebo eye and drug eye during 60-minute dilatation phase.

	MINUTES									
	0	1	5	10	20	30	40	50	60	
Drug	4.15 ± 0.72	4.19 ± 0.73	4.00 ± 0.73	4.54 ± 0.82	5.41 ± 0.76	5.85 ± 0.59	5.95 ± 0.51	6.00 ± 0.51	6.01 ± 0.54	
Placebo	4.25 ± 0.73	4.17 ± 0.69	3.93 ± 0.69	4.34 ± 0.57	5.11 ± 0.76	5.67 ± 0.58	5.79 ± 0.51	5.84 ± 0.68	5.90 ± 0.48	
Mean Difference	-0.10 ± 0.07	0.02 ± 0.09	0.07 ± 0.08	0.20 ± 0.09	0.30 ± 0.09	0.18 ± 0.07	0.16 ± 0.05	0.16 ± 0.08	0.11 ± 0.05	
Paired t statistic	-1.40	0.18	0.98	2.27	3.30	2.10	3.31	2.10	2.41	
P value	0.168	0.860	0.336	0.028	0.002	0.011	0.002	0.041	0.020	

Note: *Shaded boxes indicate a significant mean difference in the pupillary size between eyes instilled with drug and placebo respectively.

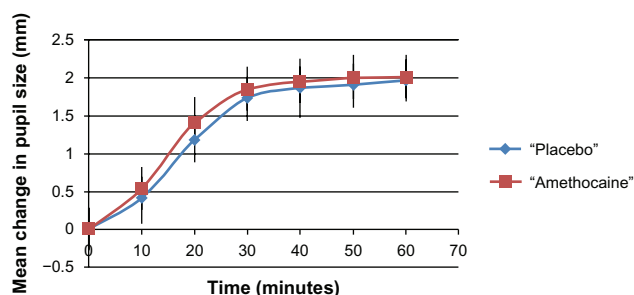


Figure 5. Rate of tropicamide-induced mydriasis with amethocaine (red) versus placebo (blue).

the eyes not reaching the CED. The time taken for 50% of amethocaine-treated eyes (ie, median time) to reach the CED of 6 mm was approximately 40 minutes. However, less than 50% of placebo-treated eyes reached the CED of 6 mm.

Magnitude of dilatation was calculated as the mean difference between pupil size at each time interval and the pupil size when tropicamide was instilled. The magnitude of pupillary dilatation achieved in the placebo-treated eyes and amethocaine-treated eyes is illustrated. The mean dilatation observed in the first 10 minutes was 0.41 ± 0.57 mm in the placebo-treated eyes (range -0.76 to 1.62 mm) as compared with a mean dilatation of 0.53 ± 0.57 mm in the amethocaine-treated eyes (range -0.68 to 1.90). The mean dilatation observed at the end of the dilatation phase (at 60 minutes) was 1.97 ± 0.60 mm in the placebo-treated eyes (range 0.75 – 3.17 mm) compared with 2.01 ± 0.61 mm in the amethocaine-treated eyes (range 0.91 – 3.22 mm). It was observed, however, that although the maximum change in pupil size occurred at 60 minutes in the placebo-treated eyes (3.17 mm), the maximum change in pupil size was recorded at 40 minutes in the amethocaine-treated eyes (3.31 mm).

No adverse events were recorded in the 50 subjects used for this study, and the drugs appear to have been well tolerated by individual subjects with only minimal to moderate discomfort felt at the time of instillation.

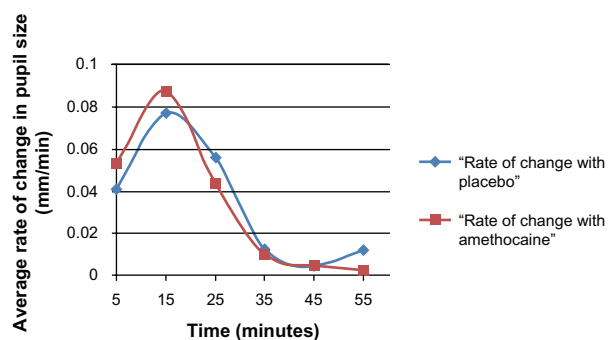


Figure 6. Average rate of change in pupil size (dilatation) in mm/min in placebo versus amethocaine-treated eyes.

Table 2. Proportion of eyes that achieved the CED of 6.0 mm in placebo eyes compared with intervention eyes.

		CED ACHIEVED IN DRUG EYE		TOTAL	
		YES	NO		
CED achieved in placebo eye	Yes	No. (%)	20 (40.0%)	3 (6.0%)	23 (46.0%)
	No	No. (%)	11 (22.0%)	16 (32.0%)	27 (54.0%)
Total		No. (%)	31 (62.0%)	19 (38.0%)	50 (100.0%)

Discussion

Darkly pigmented irides do not dilate as fast or as widely as light-colored irides. Chen and Poth attributed the reduced effect of mydriatics to reduced absorption in the iris crypts, because of mechanical obstruction by pigments contained within the chromophores.¹³ On the other hand, Angenent and Koelle explained this difference on the basis of enzymatic hydrolysis of the adrenergic mediator of pupillary dilatation by DOPA-oxidase, thereby leaving an imbalance that results in predominant function of the cholinergic sphincter in darkly pigmented irides.¹⁴ The typical Nigerian patient is black (dark-skinned) with densely pigmented dark brown irides, and therefore does not dilate quickly and sometimes does not dilate optimally for routine examination in clinical practice. Emiru noted that the dark-skinned African did not achieve the large pupil diameters of the Caucasian even after 60 minutes of dilatation.¹⁵ His reported mean pupil diameter at 60 minutes was 6.21 mm, which is comparable to the mean diameter of 6.01 ± 0.54 mm obtained at 60 minutes in the amethocaine-treated eye in this study. It must be noted however that the pupil size reported in Emiru's study may have been overestimated, as he used the subjective pupil ruler.¹⁵ Regardless of the absolute accuracy of the measurement, this comparison shows that even with an apparent potentiating agent, pupillary dilatation remains difficult in dark irides.

Ghose et al. studying the potentiating effect of 4% lignocaine on tropicamide-induced mydriasis in Indians with dark brown irides obtained a mean maximum pupil size of 6.75 ± 0.80 mm in the intervention eye and 6.08 ± 0.97 mm in the control eye.¹ This is much larger than the pupil sizes of 6.01 ± 0.54 mm and 5.90 ± 0.48 mm, obtained for amethocaine-treated and control eyes, respectively, in this study. The difference recorded in maximum pupil size, between both studies, was about 0.74 mm in the drug-treated eye and 0.18 mm in the control eye. The much larger difference in the drug-treated eye suggests that 4% of lignocaine may have a greater potentiating effect on tropicamide than 0.5% of amethocaine, or it may simply reflect a difference in the density of iris pigmentation in the two groups (Indians vs. Nigerians) as suggested by Emiru, who states that iris pigmentation and not race is more likely

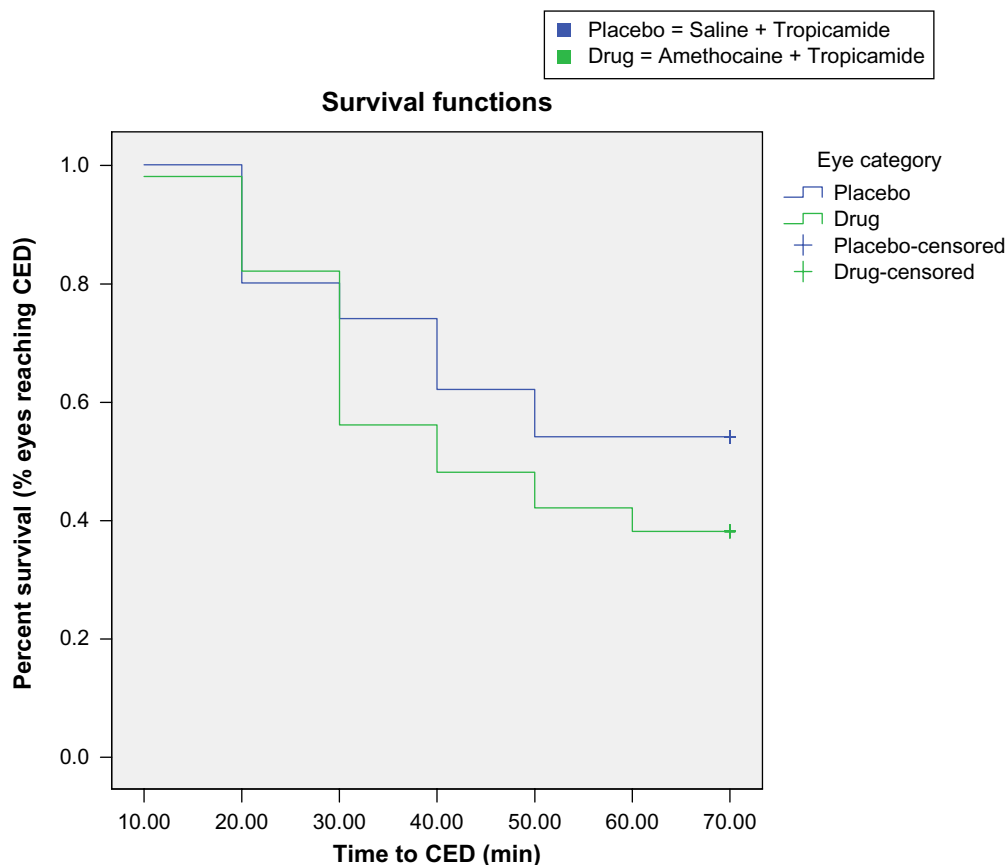


Figure 7. Kaplan-Meier Curve showing the time taken to achieve CED.

to be responsible for the poorer pupil dilatation in Africans.¹⁵ In addition, it should also be noted that the study by Ghose et al. is also potentially overestimated as they too employed the pupil ruler and not a sensitive and reliable instrument like the infrared video pupillometer.¹

Furthermore, we observed that there was a 3.7 times greater likelihood, for the eye that was pre-treated with amethocaine to achieve the CED, of 6.0 mm, than it was in the eye that did not receive amethocaine (placebo-treated or control eye), when comparing the 11 subjects who achieved CED in the amethocaine-treated eye alone to the 3 subjects who achieved CED in their placebo-treated eye alone. However, the *P* value of 0.057, using McNemar's test, was not statistically significant. This would mean that it is difficult for darkly pigmented irides to reach the CED irrespective of the pre-instillation of an anesthetic.

Topical anesthetics like amethocaine are known to inhibit the rate of corneal epithelial cell migration by disrupting cytoplasmic action in filaments and destroying superficial corneal epithelial microvilli.¹⁶ The mechanism of amethocaine's potentiating effect on tropicamide-induced mydriasis is believed to be because of an increase in the intraocular penetration of the tropicamide as a result of greater absorption through the microscopic disruptions in the corneal epithelium and reduced tearing, which increases contact time and by extension, the

bioavailability.^{1,17–20} While most authors would agree that both light and dark irides demonstrate a potentiated mydriasis when pre-treated with amethocaine, Siderov reported a potentiating effect only in light-colored irides and not in darkly pigmented eyes.² Furthermore, Haddad et al. claimed there was no potentiating effect at all.²¹ The results of this study, however, clearly suggest a potentiating effect of the anesthetic on dilatation, in the eyes pre-treated with amethocaine, even though the difference was not marked clinically. Siderov et al. may have failed to record any potentiating effect using just a magnifying loupe and ruler in the darkly pigmented eyes because his method of measuring was less sensitive to the small changes in pupil size.² In a different paper studying the effect of dosage on the mydriatic efficacy of tropicamide, Siderov and Nurse reported an increased mydriasis with two drops of tropicamide as compared with a single drop in control eyes.²² It is therefore likely that the potentiating effect may be amplified with an increase in the dosage or concentration of the anesthetic or mydriatic. The infrared pupillometer overcomes this limitation in two ways. The infrared LEDs capture the image of the pupil in high contrast (black pupil on a white (iris) background) as shown in Figure 1, making the measuring points easier to identify by the software. Also, because the video camera measures to an accuracy of 0.03 mm, even small changes in pupil size were accurately recorded. Though



the sample size was small and may be considered a potential limitation of this study, the sensitivity and the accuracy of the video pupillometer reduced the margin of error.

In conclusion, this study demonstrated that an initial drop of amethocaine had a statistically significant effect on the rate and amplitude of tropicamide-induced pupillary dilatation in darkly pigmented irides, though the change was small and was not clinically overt. This relatively modest change is related to the fact that darkly pigmented pupils are generally difficult to dilate,^{15,23–26} however, it does not mean that this small effect cannot influence clinical diagnoses or therapies. We therefore submit that pre-instillation of amethocaine does appear to potentiate the mydriatic effect of tropicamide in darkly pigmented African irides. We suggest that this evidence supports the routine use of an anesthetic before dilating patients with tropicamide; it is safe to use and does not add any additional burden to the patient. This may have potential clinical benefit where inadequate pupillary dilatation may negatively influence the outcome of the ophthalmic procedures such as cataract surgery, NdYAG or femtosecond laser capsulotomy, pan-retinal photocoagulation, fundus photography, and other retinal imaging or examination techniques. It is probable that greater effects may be observed by increasing the dosage or concentration of either the anesthetic or mydriatic or both drugs. Further studies to assess the effect of larger doses or concentrations of the anesthetic and mydriatic are recommended.

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Author Contributions

Conceived and designed the experiments: OAO. Analyzed the data: OAO. Wrote the first draft of the manuscript: OAO. Contributed to the writing of the manuscript: JWO. Agree with manuscript results and conclusions: BGKA, AOA. Jointly developed the structure and arguments for the paper: OAO, JWO. Made critical revisions and approved final version: JWO, AOA, BGKA. All authors reviewed and approved of the final manuscript.

DISCLOSURES AND ETHICS

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance

with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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