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Aging and topical pilocarpine concentrations effects on pupil size and tear flow rate

Michael O. Emina

Department of Optometry, University of Benin, Benin City, Nigeria

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KEYWORDS
Pilocarpine;
Tear secretion;
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Abstract
Purpose: This is to evaluate the effects of age and different concentrations of pilocarpine on tear flow rate and pupil size.
Methods: The tear flow rates and pupil sizes of eighty-one volunteers were measured with Schimer's strips and a meter rule gauge, respectively. These procedures were also employed in testing the effects of 2% and 4% concentrations of pilocarpine on tear secretion and pupil constriction.
Results: There were significant differences between the initial mean tear flow rate and the mean tear flow rates after the installation of 2% pilocarpine (critical $t = 1.96$ and calculated $t = 6.46$) and 4% pilocarpine (calculated $t = 8.83$). There were no significant differences in the initial tear flow rates between the sexes. There were significant differences between the mean tear flow rates obtained with 2% and 4% concentrations of pilocarpine (calculated $t = 3.41$). The mean initial tear flow rate for the eighty-one volunteers was 13.84 mm ± 1.19 (SD).There were significant differences between the mean initial pupil size 2.96 mm ± 1.04 (SD) and the mean pupil sizes 2.38 mm ± 1.11(SD) after the instillation of 2% pilocarpine (Critical $t = 1.96$ and calculated $t = 2.46$).
Conclusion: The basal tear flow rate decreased with advancing age and topical pilocarpine increased tears secretion in all the age groups. The age groups (31 years and above) were more tolerant to the stinging effect of pilocarpine and induced reduced pupil sizes, but there were no significant differences between the mean pupil sizes obtained with the pilocarpine concentrations.

*Corresponding author. Department of Optometry, University of Benin. P.M.B. 1154, Benin City, Nigeria
E-mail address: oemina@yahoo.com (M.O. Emina).

PALABRAS CLAVE
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Cloruro de Benzalcomio;
Epiforia

Efectos del envejecimiento y de las concentraciones de pilocarpina tópica sobre el tamaño pupilar y la tasa de secreción lagrimal

Resumen
Objetivo. Evaluar los efectos del envejecimiento y de diferentes concentraciones de un colirio de pilocarpina sobre la tasa de secreción lagrimal y el tamaño pupilar.
Métodos. Se determinaron las tasas de secreción lagrimal y el tamaño pupilar en 81 individuos voluntarios mediante una tira de Schirmer y un pupilómetro junto con una regla transparente milimetrada, respectivamente. Estos procedimientos también se usaron para analizar los efectos de concentraciones de un colirio de pilocarpina al 2 y al 4% sobre la secreción lagrimal y la constricción pupilar.
Resultados. Se identificaron diferencias significativas entre la tasa media inicial de secreción lagrimal y las tasas medias después de la instilación de colirio de pilocarpina al 2% (t crítica = 1,96 y t calculada = 6,46) y al 4% (t calculada = 8,83). No hubo diferencias significativas en las tasas de secreción lagrimal inicial entre sexos. Se detectaron diferencias significativas entre las tasas medias obtenidas con la concentración de pilocarpina al 2 y al 4% (t calculada = 3,41). La tasa inicial media de secreción lagrimal de los 81 individuos fue de 13,84 mm ± 1,19 (DE). Hubo diferencias significativas entre el tamaño pupilar inicial medio (2,96 mm ± 1,04 [DE]) y el tamaño pupilar medio (2,38 mm ± 1,11 [DE]) tras la instilación de colirio de pilocarpina al 2% (t crítica = 1,96 y t calculada = 2,46).
Conclusión. La tasa basal de secreción lagrimal disminuyó con la edad avanzada y el colirio de pilocarpina aumentó la secreción lagrimal en todos los grupos de edad. Los grupos de edad de 31 años en adelante fueron más tolerantes al escozor provocado por la pilocarpina y la disminución inducida del tamaño pupilar, pero no hubo diferencias significativas entre los tamaños medios obtenidos con las diferentes concentraciones de pilocarpina.

Introduction

Pilocarpine eye drop is mainly employed in the treatment of glaucoma and it causes pupil constriction. The eye drop causes temporary blur vision that last for 2 to 3 hours following topical instillation.[1] Pilocarpine, a tertiary amine, belongs to a group of drugs called parasympathomimetics and was reported to be available in concentrations exceeding 4%. However, there was usually no advantage in using such higher concentrations except in black patients and in patients with very dark pigmented rides.[2,3] Topical pilocarpine concentrations higher than 4% are not readily available in Nigeria. Therefore 2% and 4% concentrations produced by Martindale Pharmaceuticals Limited, England, were purchased for this study because of their availability.

Oral and intravenously injected pilocarpines had been employed in the stimulation of salivary and lacrimal glands for higher secretions. They were found useful in the treatment of dry eye patients, but had some measures of systemic adverse effects.[4-6] Thus pilocarpines served as a temporary remedy in alleviating the sufferings of dry-eyed patients. Tsifeteki et al.[6] employed oral pilocarpine for their study and found significant improvement on dry eye patients, but did not substantiate if there was an increase in tear secretion. Tomlison et al.[7] reported that the volume of tears in the eye was dependent on the rate of production, elimination, drainage and evaporation of tears. From these reports it could be deduced that advancing age might be a factor to be considered. Masahiko and Hsiao-Fu[8] revealed that the rate of tear secretion decreased with advancing age. Lambert et al.[9] argued that there was no decrease in tear production with advancing age and their result revealed no significant differences between the males and the females in the study. However, they[9] observed 40% reduction in the mean tear flow rate (MTFR) measured with Shirmer's strip before and after the application of anesthetic. Several authors[10-12] reported that the prevalence of dry eye increased progressively with age, and individuals between 31 to 40 years and above age were at a higher risk.

The effects of topically instilled pilocarpine on tear flow rate and pupil size have not been extensively documented and topical pilocarpine has for a long time been employed in the treatment of glaucoma patients with no major systemic side effect. Topical pilocarpine might be useful as a temporary remedy for tear volume related dry eyes, if found to stimulate sustained tear secretion. Therefore the effects of topical pilocarpine concentrations on pupil size and tear flow rate of healthy eyes of black Africans in different groups of age and gender were determined.

Methods

Teenagers and adults between 10 to 50 years of age were recruited from the population of University of Benin, Benin city, Nigeria. This study on human subjects was conducted in accordance with the tenets of the declaration of Helsinki. Verbal and written consents were obtained from the volunteers, after explanation of the project procedures. All potential volunteers underwent partial eye examination in

order to rule out ocular infections, dry eye symptoms and significant refractive errors (errors more than ± 1.5 DS, including the spherical equivalent of the cylinder lens). Volunteers with suspected epiphoria, binocular problems, observable ocular diseases and reports of drug allergies or sensitivity to the preservative Benzalkonium chloride were eliminated from the study. A total of 140 volunteers were examined for the project, but 81 participants, consisting of 51 (63%) males and 30 (37%) females met the selection criteria. The range of age of the 81 selected subjects was between 10 and 45 years and the subjects were divided into four groups of age: 10-20, 21-30, 31-40, and 41-50 (Table 1).

Two independently trained persons graded the iris colours of the participants by employing Seddon et al.[13] methods of classification, using the set of available standard photographs. Iris was graded under natural daylight as the subjects fixate at distant object. For this study, grades 1 to 3 were considered as light iris colour, while grades 4 and 5 were considered as brown to dark iris.

All the participants that met the criteria for selection had brown to dark irises. The vertical pupil diameter size of the participant's right eye was measured with a hand held gauge marked in millimeters, before and after the instillation of the eye drops. The vertical pupil diameter measured before the commencement of instillation of pilocarpine was the control experiment. An average of three readings of the vertical pupil diameter was recorded for each subject before and after the instillation of pilocarpine.

The volume of pilocarpine was determined by measuring a total of 1,000 drops from 5 different bottles using a micro liter syringe (100 μl). The observed mean volume of the drops from all the five bottles was 29.5 μl, ± 4.96. The mean drop volumes from the different bottles were 30.3 μl, 29.5 μl, 29.1 μl, 28. 7 μl and 29.4 μl. The droppers (10 ml) with the concentrations of pilocarpine to be tested were given to each participant for instillation of pilocarpine, one drop was applied twice daily for three days on the right eye. Therefore each individual had a measured minimum of about 28.7 μl drop volume instilled twice daily into the right eye. The participants were thought to occlude the puncta for three minutes, immediately after the instillation of one drop of pilocarpine. This precaution was adopted to avoid as much as possible direct systemic absorption of the drug. The participants were asked to visit the clinic daily between 4 pm. and 6 pm, in order to monitor their tolerance for pilocarpine and to administer the last one drop for the day. The time 4 pm to 6 pm was adopted for convenience in order to avoid busy hours of the clinic. This was also to ensure that each participant had at least one drop of pilocarpine instilled daily and for precautionary reasons. At the end of the third day between 4 pm. and 6 pm pupil sizes were evaluated and the Schirmer's strips were employed for the measurements of tear flow rates of the participants. All the subjects complied with the instructions and procedures.

The initial tear flow rates and pupil sizes of the volunteers were measured before the commencement of instillation of 2% pilocarpine for three days. The measurements of the initial tear flow rates and pupil sizes were for the control experiment. A period of two weeks of rest was allowed for each subject after the three days of administration of 2% pilocarpine. The instillation of 4% pilocaropine commenced immediately after the two weeks of rest. The procedure was repeated with 4% pilocarpine. The 2% and 4% pilocarpine concentrations employed in this study were produced by Martindale pharmaceutical in England. The eye drops were sterile solution of pilocarpine hydrochloride BP containing purified water and Benzalkonium chloride 0.01% as a preservative.

Table 1 Population distribution of gender within the groups of age

Age groups in years	Males	Females	Total
10-20	9 (17.7%)	4 (13.3%)	13 (16.1%)
21-30	22 (43.1%)	10 (33.3%)	32 (39.5%)
31-40	17 (33.3%)	14 (46.7%)	31 (38.3%)
41-50	3 (5.9%)	2 (6.7%)	5 (6.2%)
Total	51 (63%)	39 (37.0%)	81 (100%)

After three days of instilling the eye drop, measurements for tear flow rates and pupil size were carried out between the hours of 4pm. and 6pm. The inferior cul-de-sac was blotted with tissue paper, in order to remove the already accumulated reflex tears, before the bent tip of the standardized Schirmer's strip was placed at the lateral 1/3 of the lower eyelid of each volunteer. The volunteer being tested with Schirmer's strip was instructed to look forward and blink normally. The strip was removed after 5 min. and the amount of wetting (tear flow rate) was recorded in millimeters. The average of three readings was recorded for each subject during the measurement of tear rates and pupil sizes. The pupil sizes and the tear flow rates measurements obtained for each group of age were presented in means and standard deviations. The statistical significance between controls and trials was estimated using two tailed *t* test set at 0.05 confidence level and $P < .05$ was considered significant.

Results

In the study population, the average age for the males was 28.59 ± 7.51 (SD) years and the mean age for the females was 29.89 ± 7.01 (SD) years. The mean age for the total population examined was 29.06 ± 7.34 (SD) years. The mean basal (or initial) tear flow rate (IMTFR) for the eighty-one volunteers was 13.84 mm ± 1.20(SD). Many of the volunteers selected were between 21 and 40 years of age as shown in Table 1.

The initial mean tear flow rate (IMTFR) value for the female participants was 13.48 ± 1.34 (SD) and the males had initial mean tear flow rate (IMTFR) of 13.98 mm ± 1.22 (SD). There were no significant differences between the initial tear flow rates (IMTFR) of the male and the female participants (critical $t = 1.96$ and calculated $t = 1.72$), using two-tail *t* test, at 0.05 confidence level.

The initial mean tear flow rate (IMTFR) values for the different groups of age decreased with increase in age in both the males and the females. In Table 2, the differences between the mean tear flow rates (MTFR) for different groups of age and genders were reduced after applying two and four percent concentrations of pilocarpine. There were

no significant differences between the males and the females tested with either 2% or 4% pilocarpine concentrations (Critical $t = 1.96$, calculated t for 2% pilocarpine = 1.41 and calculated t for 4% pilocarpine = 0.83), using two-tailed t test. The mean tear flow rate value for males in the group of age between 10-20 years was higher in proportion than that observed in the females in the same group; this trend was observed in all the groups of age, but had no statistical significance. The observed differences in the mean tear rate values between the genders in the different groups of age were maintained before and after the administration of the pilocarpine concentrations. The subjects complained about slight transient blurred vision after the topical instillation of pilocarpine into the right eye. However, there was no other serious complaint from the participants except for the slight red eye and stinging experience after the instillation of pilocarpine.

The initial mean tear flow rate (IMTFR) for the participants was 13.84 mm ± 1.20 (SD) and the mean tear flow rate value after the application of 2% pilocarpine was 15.08 mm ± 144 (SD). There were significant differences between the mean initial tear flow rate value and the mean tear flow rate value after the instillation of 2% pilocarpine (Critical $t = 1.96$ calculated $t = 6.46$), using t test at 0.05 confidence level. The mean tear flow rate value for 4% pilocarpine was 15.87 mm ± 2.00 (SD). There were significant differences between 2% and 4% concentrations of pilocarpine (critical $t = 1.96$ and calculated $t = 3.4$) (Table 3).

The participants in the group of age 10 to 20 years had higher initial mean tear flow rate (IMTFR) values than others in the groups of age between 31 to 50 years. The initial mean tear flow rate of the participants in the 10 to 20 group of age was compared statistically with the IMTFR of 41 to 50 years group of age and it was found that there were significant differences between the two groups of age (Critical $t = 1.71$ and calculated $t = 3.08$), using two- tailed t test at 0.05 confidence level. The initial mean tear flow rate value 14.25 mm ± 0.48 of volunteers in the 10 to 20 years group of age was similar to the value 14.32 ± 1.33 observed after the treatment with 2% pilocarpine of volunteers in the age group 41 to 50 years; there were no statistical significant differences between the two values (critical $t = 1.96$ calculate $t = 0.18$), using two- tailed t test, at 0.05 confidence level. The mean tear flow rate (MTFR) values obtained after treatment with 4% pilocarpine for the groups of age 10-20 and from 41 to 50 were 16.26 mm ± 1.73 and 15.21 mm ± 1.76, respectively. There were also no significant differences between the mean tear flow rates of the volunteers in groups of age 10 to 20 years and 41 to 50 years after the instillation of 4% pilocarpine (Critical $t = 1.96$, calculated $t = 1.21$), using two-tailed t test statistics.

There were no significant differences in the mean pupil sizes between 2% and 4% pilocarpine concentrations after the instillations (calculated $t = 1.059$, critical $t = 1.96$), using two tailed t test at 0.05 confidence level. However, there were significant differences between the mean initial pupil size 2.93 mm ± 1.04 (SD) and the mean pupil size after the instillation of 2% pilocarpine 2.3 mm ± 1.11(SD), (critical $t = 1.96$ and calculated $t = 2.46$), using t test statistics (Table 4).

Discussion

There are two kinds of tears production, which can be distinguished from each other, the basic and reflex secretion. The diagnostic tests presently available are limited mainly

Table 2 Mean tear flow rate (MTFR) for the different groups of age and gender

Age group in years	Initial mean tear flow rates for (IMTFR)		Mean tear flow rate (MTFR) with 2% pilocarpine for		Mean tear flow rate with 4% pilocarpine for	
	Females	Males	Females	Males	Females	Males
10-20	14.00 ± 1.12	14.48 ± 0.7	14.32 ± 2.31	15.80 ± 0.82	15.21 ± 2.92	16.63 ± 0.97
21-30	13.00 ± 1.08	13.68 ± 1.91	14.56 ± 1.27	15.25 ± 1.60	15.61 ± 1.38	16.00 ± 1.67
31-40	13.88 ± 1.09	13.93 ± 1.07	15.14 ± 1.46	14.92 ± 1.50	16.04 ± 1.88	15.71 ± 1.47
41-50	12.50 ± 0.71	12.60 ± 1.57	13.88 ± 0.177	14.50 ± 1.58	14.25 ± 0.35	15.55 ± 2.02
Total MTFR	13.48 ± 1.34	13.94 ± 1.22	14.45 ± 1.35	14.96 ± 1.62	15.26 ± 1.66	15.82 ± 1.45

Table 3 Mean tear flow rate with 2% and 4% pilocarpine in different groups of age

Age group rate in years	Initial mean tear flow rates	Mean tear flow rate with 2% pilocarpine	Mean tear flow with 4% pilocarpine
10-20	14.25 ± 0.48	15.45 ± 1.44	16.26 ± 1.73
21-30	13.47 ± 1.50	14.96 ± 1.47	15.81 ± 1.51
31-40	13.90 ± 1.06	15.02 ± 1.46	15.86 ± 1.64
41-50	12.57 ± 1.27	14.32 ± 1.33	15.21 ± 1.76
Total	13.84 ± 1.20	14.95 ± 1.43	15.79 ± 1.66

Table 4 Mean pupil sizes before and after instillations of 2% and 4% pilocarpine

Age group i n years	Initial mean pupil size	Mean pupil sizes with 2% pilocarpine	Mean pupil sizes with 4% pilocarpine
10-20	3.86 ± 0.48	2.85 ± 1.44	2.65 ± 1.73
21-30	2.68 ± 1.33	2.27 ± 1.47	2.25 ± 1.61
31-40	2.64 ± 1.06	2.18 ± 1.36	2.20 ± 1.64
41-50	2.64 ± 1.27	2.22 ± 1.33	2.21 ± 1.26
Total mean	2.96 ± 1.04	2.38 ± 1.11	2.39 ± 1,45

to approximately determining tears secretion.[14] The production of tears from the lacrimal gland, tear distribution over the cornea by blinking, evaporation of tears from the ocular surface and drainage through the nosolacrimal duct are the processes that tears undergo.[15] The quantity of tears on these tissues at any given time is essential for ocular health. Abnormalities in the course of the tear film may result in eye conditions that can be detrimental to vision. Therefore the production or the stimulation of the lacrimal gland to produce more tears may be an essential step in addressing tears related problems. Thus pilocarpine was employed to stimulate tears secretion.

The major complaints after the application of the parasympathomimetic agents were constricted pupil, red eyes and stinging sensation and were observed in higher proportion in the groups of age between 10 to 30 years of age. Transient blurred vision, and reduced pupil sizes in the volunteers as a result of the drugs were common observations among the participants. There was no other major adverse effect of pilocarpine observed during the period of study. However, subjects above 32 years of age showed slight reduction of their pupil sizes and were more tolerant or had no stinging effect of the drug. There were no significant differences between the mean pupil sizes observed after the instillation of 2% and 4% pilocarpine concentrations (Critical $t = 1.96$ and calculated $t = 1.83$), using two tailed t test at 0.05 confidence level.

The mean (initial) basal tear flow rate showed no significant differences between the male and the female participants (Critical $t = 1.96$ and calculated $t = 1.72$). The study revealed significant differences between the initial mean tear flow rate (IMTFR) value and the mean tear flow rate value after the administration of 2% pilocarpine. (Critical $t = 1.98$ and calculated $t = 6.46$), using t test statistics. There were also significant differences between 2% and 4% pilocarpine mean tear flow rate values. (Critical $t = 1.96$ and calculated $t = 3.41$). Therefore, there was an increase in the observed tear flow rate with topical pilocarpine. This observation was similar to the one reported by Vivino et al.[4] that pilocarpine increased the secretion of tears. There were no observed significant differences between the males and females tear flow rates in the study. Lambert et al.[9] also observed no significant differences in the mean tear flow rate values between the males and the females.

The mean tear flow rate values obtained after the instillation of 4% pilocarpine revealed no significant differences between the subjects in 10 to 20 and 40 to 50 years groups of age. This showed that 4% pilocarpine bridged the gap between the mean tear flow rate values of the youths (10 to 20 years) and the older adults (31-50 years). Although there were no significant differences between 2% and 4% concentrations of pilocarpine in stimulating tear production. It was observed that the mean tear flow rates were adequate (14.25 mm) for all the age groups after the instillation of 4% pilocarpine.

The initial mean tear flow rate values revealed that the group of age from 10 to 20 years had higher IMTFR values than subjects in the 41 to 50 years group and the differences were statistically significant (Critical $t = 1.71$ and calculated $t = 3.08$), using a two-tailed t test statistics. This agreed with the study carried out by Zintz and Schilling[16] and other[10-12,14,17] on the relationship between tear secretions with age. The differences in the initial mean tear flow rate values for the group of age from 10 to 20 years and the mean tear flow rate values obtained after the instillation of 2% pilocarpine in the group of age from 41 to 50 were statistically negligible (Critical $t = 1.96$ and calculated $t = 0.18$). Therefore, 2% pilocarpine may be adequate in stimulating increased tear production particularly in the groups of age from 31years and above.

It may be necessary to carry out further studies on the stimulating effects of topical pilocarpine on tear secretion and on dry eye patients, resulting from insufficient tear secretion. However, in this study, it can be concluded that tear flow rate decreases with advancing age and topical pilocarpine is useful in increasing the tear flow rate in the aging healthy eye of the black population.

Conflict of interest

The author states he has no conflict of interest.

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