Effects of Topical Timolol Maleate on Anterior Segment Structure, Pupil Dynamics and Retinal and Choroidal Thickness in Healthy Subjects

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ABSTRACT

Purpose: To investigate the effect of a single dose of topical timolol maleate 0.5% on anterior segment structure, pupil dynamics, and retinal and choroidal thickness in healthy subjects.

Materials and Methods: Thirty subjects were included in this prospective and observational study. One eye of the participants received a single dose of topical benzalkonium chloride (BAC) preserved Timolol maleate 0.5% (Timosol 0.5%, Bilim Ilac, Istanbul, Turkey) (treated eye). Contralateral eye received a single dose of sodium hyaluronate ophthalmic solution without preservative (control eye). Main outcome measures were central corneal thickness (CCT), aqueous depth, anterior chamber volume, iridocorneal angle, horizontal anterior chamber diameter, static and dynamic pupil functions, central macular thickness, and choroidal thickness.

Results: Mean CCT was significantly higher after timolol instillation in treated eyes ($p=0.001$). Timolol did not affect aqueous depth, anterior chamber volume, iridocorneal angle and horizontal anterior chamber diameter in treated eyes ($p>0.05$ for each). Mean pupil diameters were similar under scotopic, mesopic and photopic conditions before and after timolol instillation ($p>0.05$ for each); however, pupil dilation speed was significantly lower after timolol instillation in the treated eyes ($p<0.05$). Mean central macular thickness was significantly higher after timolol instillation in treated eyes ($p=0.005$); however, no significant difference was observed in choroidal thickness ($p>0.05$). Pre- and post-instillation outcomes were similar in control eyes for all parameters ($p>0.05$ for each).

Conclusion: Topical BAC-preserved timolol maleate reduces pupil dilation speed without any effect on static pupil functions and increases the CCT and central macular thickness.

Key Words: Pupillometry, Timolol, Retinal thickness, Choroidal thickness, Cornea.

INTRODUCTION

Timolol maleate is a non-selective, beta-adrenergic blocker and the mainstay drug for the first-line treatment of glaucoma, either as a monotherapy or in combination with other drugs.[1] Timolol maleate lowers intraocular pressure and suppresses aqueous humor production by lowering cyclic adenosine monophosphate levels via beta-adrenergic receptor blockage and Cl^--HCO_3^-exchange and Na^+-K^+-Cl^- cotransporters inhibition in the ciliary epithelium.[2-4] Timolol maleate binds to all types of beta-adrenergic receptors; correspondingly, it may affect several ocular structures that contain beta-adrenergic receptors other than the intended target. The epithelium and endothelium of the cornea, iris muscles, ciliary body, trabecular meshwork, and retina pigment epithelium possess beta-adrenergic receptors.[5-8] The use of topical timolol may cause functional and structural changes in those tissues. The effect of timolol can be observed 20 minutes after administration and lasts for 24 hours.[9] However, the efficacy of timolol may vary in ocular structures due to up- and down-regulation of beta-receptor density.[10]

Tachyphylaxis is an important factor and may occur within days or months after the instillation of timolol.[11] Tachyphylaxis may mask some of the ocular effects of timolol in patients who have taken the drug over an extended period; therefore, previous study outcomes may be distorted due to this phenomenon. Additionally, large proportion of glaucoma patients have been shown to have poor adherence to their medical treatment.[12] Poor patient adherence may also increase the frequency

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of ocular side-effects of timolol that are masked due to tachyphylaxis in patients with good adherence to medical treatment. Previous studies have investigated the side-effects of timolol on patients with long term use; however, those studies did not investigate immediate ocular side effects of timolol, which possibly affects patients with poor adherence. Current knowledge regarding the effect of topical timolol should be updated in consideration of tachyphylaxis.

We hypothesize that a single dose of timolol maleate may arise ocular side-effects that are not experienced in regular use due to tachyphylaxis. The present study aimed to assess the effect of a single dose of timolol maleate 0.5% eye drop on the anterior segment structure, pupil functions, and retinal and choroidal thickness in healthy subjects.

MATERIAL AND METHODS

This prospective and observational study was conducted at ophthalmology department of Mustafa Kemal University Faculty of Medicine, Hatay, Turkey, in accordance with the ethical standards of the Declaration of Helsinki. The study protocol was approved by the institutional board of the Mustafa Kemal University Faculty of Medicine ethics committee. All participants provided written informed consent prior to undergoing all examinations.

All included subjects were healthy and had no history of systemic or ocular disease. Subjects with the following conditions were excluded: corrected distance visual acuity <20/20 in the Snellen chart, myopia or hyperopia greater than 0.5 diopter, history of ocular trauma or surgery, corneal diseases, uveitis, glaucoma, pseudoexfoliation syndrome, anterior or posterior synechiae, cataract, congenital or acquired iris and pupil anomalies, retinal diseases that may affect pupil functions, recent or permanent use of topical medications, and use of drugs that may affect pupil functions.

Sixty eyes of 30 healthy subjects, who met the eligibility criteria, were included in the study. The eyes of the subjects were randomized and one eye (treated eye) received one drop of benzalkonium chloride (BAC) preserved Timolol maleate 0.5% (Timosol 0.5%, Bilim Ilac, Istanbul, Turkey). The contralateral eye (control eye) received one drop of preservative free sodium hyaluronate ophthalmic solution. All subjects applied nasolacrimal occlusion for five minutes after receiving eye drops.

All clinical examinations and measurements were performed before and two hours after eye drop instillation. Same clinician (V.C.), who was blinded to randomization of eyes, performed the all ocular examinations and measurements. All subjects underwent a complete ocular examination including best corrected visual acuity test with a Snellen chart, intraocular pressure measurement, biomicroscopy of anterior segment, and fundus examination. Anterior segment parameters including central corneal thickness (CCT), anterior chamber volume (ACV), iridocorneal angle (ICA), aqueous depth (AD), and horizontal anterior chamber diameter (HACD) were measured with Sirius Topographer (CSO, Firenze, Italy). The AD corresponds to the distance from the central corneal endothelium to the anterior lens capsule. The HACD corresponds to the distance between the opposing cornea-scleral junctions at horizontal meridian.

Pupil responses were measured with the pupillometry function of the Sirius Topographer (CSO, Firenze, Italy) using Phoenix v2.1 software (Costruzione Strumenti Oftalmici, CSO, Firenze, Italy). All measurements were performed at the same time of the day (10:00–12:00 am) to avoid the circadian changes in the pupillary response.[13] All measurements were performed based on Prakash et al.'s method.[14] Pupil responses were evaluated after a five-minute dark adaptation, which was followed by measurements at scotopic (0.4 lux), mesopic (4 lux), and photopic (40 lux) conditions. LED lighting was used for illumination in the examination room, and the illumination conditions were adjusted using a photometer. The subjects were advised to look straight ahead, not at the light source, during the measurements to prevent the accommodative response. Static pupil measurements were followed by dynamic pupil measurements. Dynamic pupil measurement was started at illumination of 500 lux. After the measurement began, the illumination was switched off until the end of the session. Dynamic pupil measurement enables to monitor pupil size change during the session at illumination conditions ranging from photopic to scotopic (Figure 1).

Speed of change in pupillary diameter was calculated using the following equation: Average speed (mm/s) was the overall average speed until that time,

$$V_{average} = ([\delta\Phi_t - \Phi_{t0}] / t)$$

where $\delta\Phi$ is the difference in the pupil diameter (mm) between time (seconds) at the time of measurement and at $t = 0$.

Posterior segment parameters including central macular thickness (CMT), ganglion cell complex (GCC) thickness and choroidal thickness (CT) were measured with optical coherence tomography (Cirrus HD 4000, Carl Zeiss Meditec, CA, USA) after pupil mydriasis. Only images with a signal strength ≥ 7/10 were used for measurements. GCC represents the three innermost retinal layers and GCC thickness was measured from the outer edge of

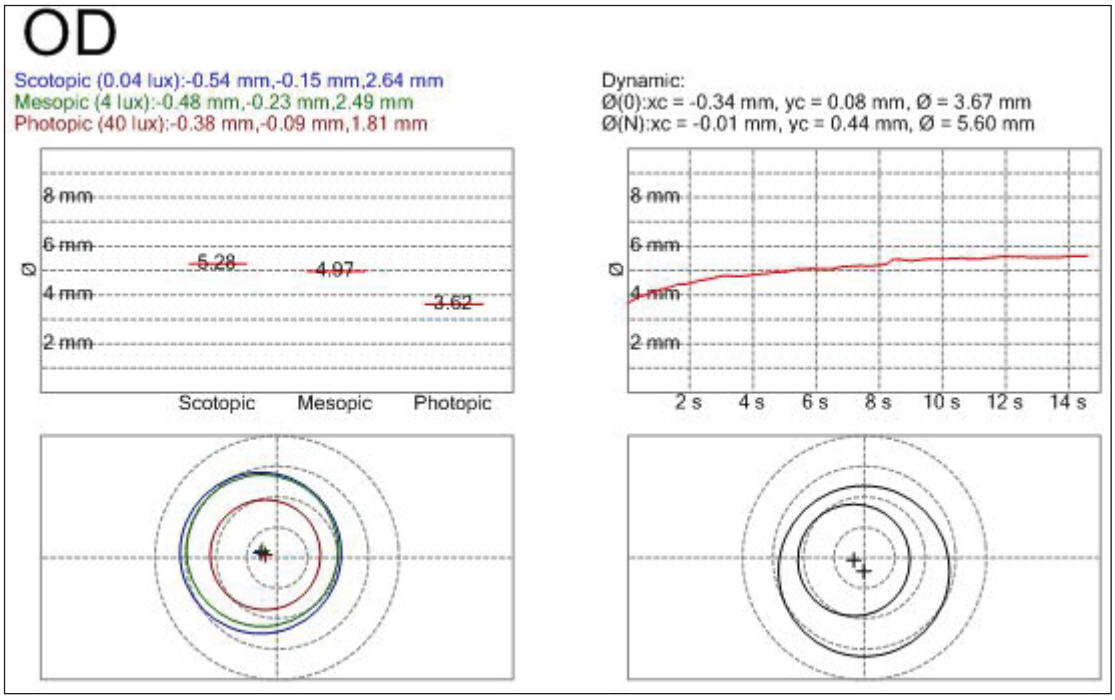

Figure 1. *Presents an output of pupillometry analysis of Sirius Topographer (CSO, Italy). The static pupil responses under different illumination conditions and pupil diameters are shown on the left side of the output graph. The dynamic pupil responses and pupil diameters at a particular time are shown on the right side of the output graph.*

the hyperreflective internal limiting membrane to the outer edge of the hyporeflective inner plexiform layer. GCC thickness measurement was performed at central macular area. CT was measured from the outer edge of the hyperreflective retinal pigment epithelium to the inner sclera. CT measurement was performed at the subfovea, 3 mm nasal to the subfovea and 3 mm temporal to the subfovea.

Statistical analyses were performed with SPSS Statistics (Version 22.0, Armonk, NY: IBM Corp). The assumption of normal distribution of data was tested by the Shapiro-Wilk test. Differences in the outcomes before and after eye drop instillation were tested with paired samples t-test and Wilcoxon Signed Rank test. Differences in dynamic pupil outcomes at a particular measurement time before and after eye drop instillation were tested with paired samples t-test. A level of $p<0.05$ was assumed statistically significant for all tests.

RESULTS

Sixty eyes of 30 healthy subjects who met the eligibly criteria were included in the study. The mean age was 26.4±4.5 years (min: 20, max: 36). Of the 30 patients, 15 were male and 15 were female. Table 1 shows the mean outcomes of anterior segment parameters before and after eye drop instillation and a comparison within treated and control eyes. The mean CCT was significantly higher in treated eyes after timolol eye drop instillation, where no significant difference was observed between the pre- and post-instillation outcomes of AD, ACV, ICA, and HACD. Outcomes of all anterior segment parameters before and after eye drop instillation were similar in the control eyes.

Table 2 presents the outcomes of the static (scotopic, mesopic, photopic) pupillometry analysis and a comparison of outcomes before and after eye drop instillation in the treated and control eyes. The mean pupil diameter during the static (scotopic, mesopic, photopic) pupillometry analysis had no significant difference between the pre- and post-instillation outcomes in both treated and control eyes ($p>0.05$ for each). Figure 2 presents pre- and post-instillation outcomes of pupil dilation speed instant by instant in the treated eyes and a comparison of the pre- and post- instillation outcomes. The mean pre- instillation pupil dilation speed outcomes at the 1st, 2nd, 4th, and 6th second were significantly higher than the mean post-instillation pupillary dilation speed in the treated eyes ($p<0.05$ for each). Figure 3 presents the pre- and post-instillation outcomes of pupil dilation speed instant by instant in the control eyes and a comparison of the pre- and post-instillation outcomes. The mean pre-instillation pupil dilation speed during the entire measurement period was similar with the mean post-instillation pupil dilation speed in the control eyes ($p>0.05$ for each).

Table 3 shows pre- and post-instillation outcomes of

Table 1. *Outcomes of anterior segment parameters before and after eye drop instillation.*

	Treated Eyes			Control eyes		
	Pre- instillation	Post-instillation		Pre-instillation	Post- instillation	
	Mean±SD (Min-Max)	Mean±SD (Min-Max)	P value	Mean±SD (Min-Max)	Mean±SD (Min-Max)	P value
CCT (microns)	544.2±27.1 (490 – 595)	550.5±28.5 (502 – 610)	0.001*	545.3±26.6 (497 – 595)	543.4±28.6 (489 – 604)	0.171*
AD (mm)	3.1±0.2 (2.5 – 3.6)	3.2±0.3 (2.5 – 3.6)	0.150*	3.1±0.2 (2.6 – 3.6)	3.1±0.3 2.5 – 3.7	0.854*
ACV (mm^3)	157.2±21.3 (118 – 189)	158.1±20.0 (117 – 188)	0.346*	159.0±21.2 (120 – 194)	158.2±22.5 (116 – 195)	0.338*
ICA (°)	43.8±5.2 (34 – 53)	44.5±5.3 (36 – 55)	0.140*	43.4±5.5 (34 – 53)	43.8±5.6 (33 – 54)	0.147*
HACD (mm)	12.1±0.4 (11.3 – 13.0)	12.1±0.4 (11.3 – 12.9)	0.983*	12.1±0.4 (11.3 – 13.2)	12.2±0.4 (11.3 – 13.2)	0.559*

* Paired samples t test, CCT: central corneal thickness, AD: aqueous depth, ACV: anterior chamber volume, ICA: iridocorneal angle, HACD: horizontal anterior chamber diameter

Table 2. *Outcomes of the static (scotopic, mesopic, photopic) pupillometry analysis before and after eye drop instillation.*

	Treated Eyes			Control eyes		
	Pre-instillation	Post-instillation		Pre-instillation	Post- instillation	
	Mean±SD (Min-Max)	Mean±SD (Min-Max)	P value	Mean±SD (Min-Max)	Mean±SD (Min-Max)	P value
Scotopic (mm)	6.18±0.77 (5.11 – 7.52)	6.09±0.80 (4.69 – 7.78)	0.147**	5.86±0.82 (4.22 – 7.25)	5.92±0.85 (4.40 – 7.46)	0.495*
Mesopic (mm)	5.47±0.90 (3.59 – 6.66)	5.52±0.83 (4.05 – 7.37)	0.579*	5.12±0.91 (3.50 – 6.64)	5.15±0.84 (3.95 – 6.87)	0.802*
Photopic (mm)	4.28±0.83 (2.78 – 6.01)	4.31±0.83 (2.91 – 6.20)	0.734*	4.04±0.69 (2.76 – 5.21)	4.10±0.71 (2.87 – 5.61)	0.406*

* Paired samples t test, ** Wilcoxon Signed Rank test

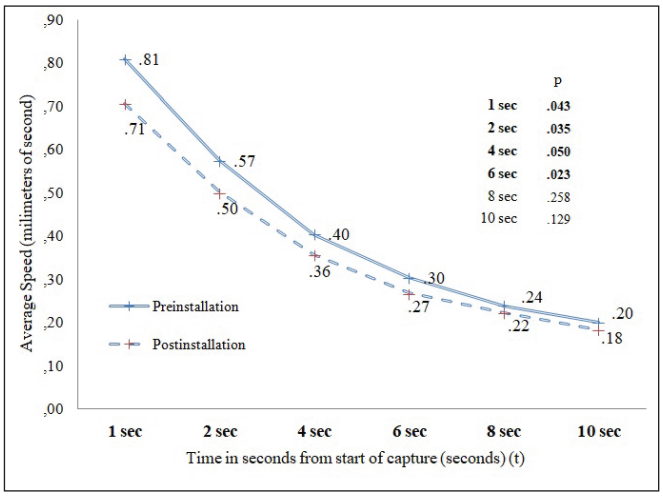

Figure 2. Shows pre- and post-instillation outcomes of pupil dilation speed instant by instant in the treated eyes and a comparison of the pre- and post-instillation outcomes.

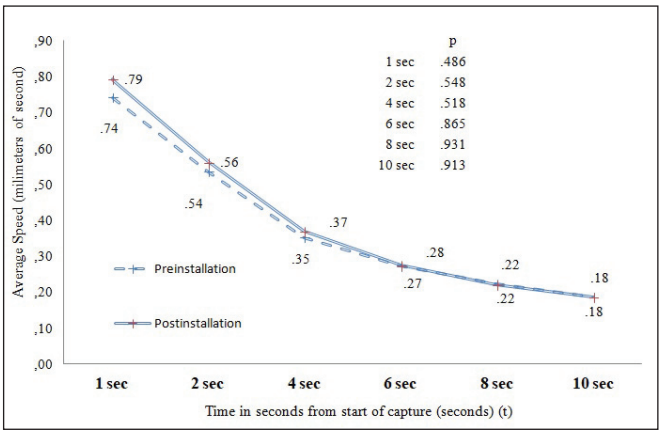

Figure 3. Shows the pre- and post-instillation outcomes of pupil dilation speed instant by instant in the control eyes and a comparison of the pre- and post-instillation outcomes.

Table 3. *Outcomes of posterior segment parameters before and after eye drop instillation.*

	Treated Eyes			Control eyes		
	Pre-instillation	Post-instillation		Pre-instillation	Post- instillation	
	Mean±SD (Min-Max)	Mean±SD (Min-Max)	P value	Mean±SD (Min-Max)	Mean±SD (Min-Max)	P value
CMT (micron)	245.9±19.9 (190 – 282)	247.5±19.7 (189 – 280)	0.005**	245.8±19.3 (191 – 284)	246.2±18.1 (200 – 283)	0.327**
GCC thickness (micron)	84.7±5.8 (74 – 97)	84.8±5.9 (75 – 100)	0.702*	84.6±5.6 (74 – 96)	84.8±5.8 (74 – 98)	0.109*
CT (fovea) (micron)	262.9±36.0 (193 – 332)	266.2±50.8 (181 – 397)	0.494*	266.8±37.9 (204 – 332)	270.8±39.4 (216 – 340)	0.440**
CT (temporal) (micron)	234.8±37.8 (169 – 311)	242.8±51.1 (152 – 365)	0.267*	242.4±44.6 (164 – 334)	249.8±41.7 (184 – 320)	0.103*
CT (nasal) (micron)	224.1±35.1 (160 – 292)	231.0±41.5 (141 – 330)	0.091*	225.5±41.1 (145 – 332)	227.7±50.6 (132 – 335)	0.631*

* Paired samples t test, **: Wilcoxon Signed Rank test, CMT: central macular thickness, GCC: Ganglion cell complex, CT: choroidal thickness

posterior segment parameters and a comparison between the pre- and post-instillation outcomes. The mean post-instillation outcome of the CMT was significantly higher than mean pre-instillation of the CMT in the treated eyes (p=0.005). No significant difference was found between the pre- and post-instillation outcomes of central macular GCC thickness and foveal, temporal and nasal CT in the treated eyes (p>0.05 for each). The mean pre- and post-instillation outcomes of all posterior segment parameters were similar in the control eyes (p>0.05 for each).

DISCUSSION

Poor medication adherence, improper drug dose or interval, and frequent discontinuation of the medication reduce the effectiveness of the drug and may trigger temporary side-effects.[15] Proper dosing and drug-free intervals are important for the drugs such as timolol because of tachyphylaxis. Beside the effect of tachyphylaxis on drug effectiveness, it may also reduce the side-effects of the drugs. Therefore, patients with poor adherence may experience different side-effects than the patients that have good adherence due to incomplete tachyphylaxis. In our study, we evaluated the effect of topical timolol on ocular structures before tachyphylaxis development and, to our knowledge, this is the first study that investigates the immediate effect of timolol before the development of tachyphylaxis in the literature.

Ciliary muscle is innervated by both parasympathetic and sympathetic neural pathways and mainly contains beta-adrenergic receptors.[16] Winn et al. showed that timolol maleate influenced accommodative response, which may cause changes in the anterior chamber structure.[16] However, consistent with our results, previous studies reported no significant change in AD, ACV, and ICA in patients under the long-term topical timolol medication.[17,18] Kim et. al reported more widening in open angle distance with timolol and brimonidine combination than timolol alone; however, exact mechanism of this phenomenon remains unexplained.[18] Vasudevan et al. reported that patients with myopia were more susceptible to prolonged accommodation decay.[19] Patients with no refractive error compromised the study groups; therefore, refractive status may mask the effect of timolol on anterior segment parameters in our study. Additionally, interindividual variability of activity of Cytochrome P450 enzyme family, which includes the systemic and ocular metabolizing enzymes of timolol, may affect the outcomes of our study results; however, we did not measure enzyme activity in our subjects.[20]

The present study revealed that topical timolol maleate increased the CCT. The corneal endothelium houses beta-adrenergic receptors, and blockage of those receptors cause a decrease in intracellular cAMP concentration, protein kinase A activity, and endothelial ion and fluid transport, which resulted in CCT thickening.[2,21,22] However, topical timolol maleate products in the market may also contain BAC, which may influence CCT through its effects on corneal permeability. Jong et al. provided significant proof regarding the toxic effects of BAC on the corneal

barrier and permeability.[23] Chen et al. showed that topical BAC increased the CCT by impairing the integrity of the corneal endothelium; however lower concentrations than 0.1% BAC did not affect CCT in rabbits.[24] The immediate increase of CCT in our study may have resulted from the cumulative effect of both the beta-adrenergic receptor blockage and BAC. Grueb et al. reported that the maximum increase in the CCT was observed 9 days after the first topical timolol maleate instillation and rapidly decreased to baseline.[25] The CCT increment is reversible in long-term timolol medication due to tachyphylaxis; however, we can speculate that noncompliance or intermittent compliance to topical timolol treatment may affect corneal integrity because of incomplete tachyphylaxis.

The dilator muscle houses mainly alpha-adrenergic receptors, and the sphincter muscle houses both alpha- and beta-adrenergic receptors.[26] Timolol has a miotic effect on animal models but not on human subjects.[27] However, Johnson et al. suggested that the dilation of the pupil was affected by timolol.[28] Slower pupil speed during dilation in our study may indicate the presence of inhibitory beta-adrenergic receptors on the sphincter pupilla, which was speculated previously in the literature.[29] Changes in pupil function may affect the contrast sensitivity and spherical aberrations causing visual impairment; therefore, immediate effect of timolol on pupil functions should be considered in patients with acute visual impairment, who have poor adherence.

In the literature, few studies have investigated the effect of topical timolol on the retina and choroid.[30,31] The blockage of beta-adrenergic receptors decreases the cAMP concentration in retinal pigment epithelium (RPE), causing an increase in net fluid absorption from the subretinal space.[32,33] However, our study revealed higher CMT in the treated eyes, whereas there was no significant change in the thickness of the GCC thickness and CT. These results indicate that the thickening of the retina resulted from outer segments of the retina, which is consistent with the knowledge of fluid accumulation in the extracellular space of the outer plexiform layer in the macular edema pathophysiology.[34] Contrary to the effect of timolol on RPE absorption, BAC has been blamed for causing macular edema, serous retinal detachment, and RPE atrophy.[35,36] Moreover, BAC triggers prostaglandin synthesis due to the wound-healing process, which may promote macular edema development.[37] Based on previous reports, to our knowledge, BAC may be the reason behind the retinal thickening in our study.

There are some limitations in our study. The sample size of the present study was limited. Therefore, the study results should be confirmed with a larger sample size. Our subjects were healthy and relatively young. The effect of timolol may not be the same in older subjects and in subjects with an ocular or systemic disorder, which means that the present study's results cannot be generalized to the entire population. Also, we could not evaluate the effects of timolol and BAC separately, therefore, separate observations should be performed in further studies.

In conclusion, our study showed that a single dose of BAC-preserved timolol maleate 0.5% significantly reduces the dilation speed of the pupil and significantly increased the CCT and CMT. Immediate effects of topical timolol should be considered in patients that have poor adherence or incomplete tachyphylaxis.

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REFERENCES

1. Uusitalo H, Kahonen M, Ropo A, et al. The evaluation of efficacy and systemic side effects of topical antiglaucoma drugs: the benefits of improved drug formulation. J Glaucoma. 1999;8:264-5.
2. Coakes RL, Brubaker RF. The mechanism of timolol in lowering intraocular pressure. In the normal eye. Arch Ophthalmol. 1978;96:2045-8.
3. Crook RB, Riese K. Beta-adrenergic stimulation of Na+, K+, Cl- cotransport in fetal nonpigmented ciliary epithelial cells. Invest Ophthalmol Vis Sci. 1996;37:1047-57.
4. McLaughlin CW, Peart D, Purves RD, et al. Timolol may inhibit aqueous humor secretion by cAMP-independent action on ciliary epithelial cells. Am J Physiol Cell Physiol. 2001;281:865-75.
5. Wax MB, Molinoff PB. Distribution and properties of beta-adrenergic receptors in human iris-ciliary body. Invest Ophthalmol Vis Sci. 1987;28:420-30.
6. Frambach DA, Fain GL, Farber DB, et al. Beta adrenergic receptors on cultured human retinal pigment epithelium. Invest Ophthalmol Vis Sci. 1990;31:1767-72.
7. Wax MB, Molinoff PB, Alvarado J, et al. Characterization of beta-adrenergic receptors in cultured human trabecular cells and in human trabecular meshwork. Invest Ophthalmol Vis Sci. 1989;30:51-7.
8. Elena PP, Denis P, Kosina-Boix M, et al. Beta adrenergic binding sites in the human eye: an autoradiographic study. J Ocul Pharmacol. 1990;6:143-9.
9. Zimmerman TJ, Kaufman HE. Timolol, dose response and duration of action. Arch Ophthalmol. 1977;95:605-7.

10. Boger III WP. Shortterm "escape" and longterm "drift.". The dissipation effects of the beta adrenergic blocking agents. Surv Ophthalmol. 1983;28:235-40.
11. Oksala A, Salminen L. Tachyphylaxis in timolol therapy for chronic glaucoma. Klin Monbl Augenheilkd. 1980;177:451-4.
12. Tsai JC. Medication adherence in glaucoma: approaches for optimizing patient compliance. Curr Opin Ophthalmol. 2006;17:190-5.
13. Zele AJ, Feigl B, Smith SS, et al. The circadian response of intrinsically photosensitive retinal ganglion cells. PLoS One. 2011;6:e17860.
14. Prakash G, Srivastava D, Suhail M, et al. Assessment of bilateral pupillary centroid characteristics at varying illuminations and post-photopic flash response using an automated pupillometer. Clin Exp Optom. 2016;99:535-43.
15. Schwartz GF, Quigley HA. Adherence and Persistence with Glaucoma Therapy. Surv Ophthalmol. 2008;53:57-68.
16. Winn B, Culhane HM, Gilmartin B, et al. Effect of beta-adrenoceptor antagonists on autonomic control of ciliary smooth muscle. Ophthalmic Physiol Opt. 2002;22:359-65.
17. Boger WP, 3rd, Puliafito CA, Steinert RF, et al. Long-term experience with timolol ophthalmic solution in patients with open-angle glaucoma. Ophthalmology. 1978;85:259-67.
18. Kim JM, Park KH, Kim CY et al. Effects of brimonidine timolol fixed combination therapy on anterior ocular segment configuration. Jpn J Ophthalmol. 2011;55:356-61.
19. Vasudevan B, Ciuffreda KJ, Gilmartin B. Sympathetic inhibition of accommodation after sustained nearwork in subjects with myopia and emmetropia. Invest Ophthalmol Vis Sci. 2009;50:114-20.
20. Volotinen M, Hakkola J, Pelkonen O, et al. Metabolism of ophthalmic timolol: new aspects of an old drug. Basic Clin Pharmacol Toxicol. 2011;108:297-303.
21. Grub M, Leitritz M, Mielke J, et al. Effect of timolol on central corneal thickness and endothelial cell density. Klin Monbl Augenheilkd. 2006;223:894-8.
22. Grueb M, Bartz-Schmidt KU, Rohrbach JM. Adrenergic regulation of cAMP/protein kinase A pathway in corneal epithelium and endothelium. Ophthalmic Res. 2008;40:322-8.
23. de Jong C, Stolwijk T, Kuppens E, et al. Topical timolol with and without benzalkonium chloride: epithelial permeability and autofluorescence of the cornea in glaucoma. Graefes Arch Clin Exp Ophthalmol. 1994;232:221-4.
24. Chen W, Li Z, Hu J, et al. Corneal alternations induced by topical application of benzalkonium chloride in rabbit. PLoS One. 2011;6:26103.
25. Grueb M, Rohrbach JM. Effect of timolol on central corneal thickness. Eur J Ophthalmol. 2013;23:784-8.
26. van Alphen GW. The adrenergic receptors of the intraocular muscles of the human eye. Invest Ophthalmol. 1976;15:502-5.
27. Wilkie DA, Latimer CA. Effects of topical administration of timolol maleate on intraocular pressure and pupil size in cats. Am J Vet Res. 1991;52:436-40.
28. Johnson SH, Brubaker RF, Trautman JC. Absence of an effect of timolol on the pupil. Invest Ophthalmol Vis Sci. 1978;17:924-6.
29. Novitskaya ES, Dean SJ, Moore JE, et al. Effects of some ophthalmic medications on pupil size: a literature review. Can J Ophthalmol. 2009;44:193-7.
30. Goto W, Ota T, Morikawa N, et al. Protective effects of timolol against the neuronal damage induced by glutamate and ischemia in the rat retina. Brain Res. 2002;958:10-9.
31. Fuchsjäger-Mayrl G, Wally B, Rainer G, et al. Effect of dorzolamide and timolol on ocular blood flow in patients with primary open angle glaucoma and ocular hypertension. Br J Ophthalmol. 2005;89:1293-7.
32. Miller SS, Hughes BA, Machen TE. Fluid transport across retinal pigment epithelium is inhibited by cyclic AMP. Proc Natl Acad Sci U S A. 1982;79:2111-5.
33. Quinn RH, Quong JN, Miller SS. Adrenergic receptor activated ion transport in human fetal retinal pigment epithelium. Invest Ophthalmol Vis Sci. 2001;42:255-64.
34. Scholl S, Kirchhof J, Augustin AJ. Pathophysiology of macular edema. Ophthalmologica. 2010;224:8-15.
35. Miyake K, Ota I, Ibaraki N, et al. Enhanced disruption of the blood-aqueous barrier and the incidence of angiographic cystoid macular edema by topical timolol and its preservative in early postoperative pseudophakia. Arch Ophthalmol. 2001;119:387-94.
36. Chou A, Hori S, Takase M. Ocular toxicity of beta-blockers and benzalkonium chloride in pigmented rabbits: electrophysiological and morphological studies. Jpn J Ophthalmol. 1985;29:13-23.
37. Makri OE, Georgalas I, Georgakopoulos CD. Drug-induced macular edema. Drugs. 2013;73:789-802.