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Original research

Efficacy of adjuvant topical timolol–dorzolamide with intravitreal bevacizumab injection in diabetic macular edema: A contralateral eye study

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Abstract

Purpose: To assess the efficacy of adjuvant topical timolol–dorzolamide with intravitreal bevacizumab (IVB) injection on anatomic and functional results in eyes affected with diabetic macular edema (DME).

Methods: In an interventional prospective contralateral pilot eye study at a third level referral academic facility, patients with bilateral DME who were treatment-naive were enrolled. Enrolled patients received a treatment plan of topical timolol–dorzolamide twice daily in the right eye. Three monthly bilateral IVB injections 1.25 mg/0.05 mL were also planned. Baseline central macular thickness (CMT) was measured by spectral-domain optical coherence tomography (SD-OCT), and clinical information such as best corrected visual acuity (BCVA) and intraocular pressure (IOP) were collected at enrollment and one month after the third injection.

Results: Eleven patients (seven females) with DME were included. BCVA and CMT improved in both eyes and IOP decreased in the right eye but did not change in the left eye. In repeated measures ANOVA analysis, the decrease in CMT and improvement in BCVA were significant in the right eye.

Conclusion: Our study suggested that adjuvant topical timolol–dorzolamide in combination with IVB may further reduce central macular thickness in eyes with DME.

Keywords: Timolol; Dorzolamide; Intravitreal bevacizumab; Diabetic macular edema; Macular thickness; Visual acuity

Introduction

Diabetic macular edema (DME) can be defined as the thickening and cystoid edema of the macula, mostly with exudate deposition that can be attributed to diabetic retinopathy. The serum glucose elevated in the course of time predisposes to a breakdown of the inner and outer retinal blood barrier. Hypoxia, oxygen-free radical accumulation, and inflammatory

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mediators lead to vascular endothelial growth factor (VEGF) expression. VEGF has a significant role in the development of DME.[1–3] VEGF is incriminated in neovascularization and increased vascular permeability. VEGF levels in both the anterior and vitreous chambers are correlated with DME severity.[4] The introduction of VEGF into a normal primate eye brings about the pathological changes of micro aneurysm formation and fosters vascular permeability, which are the characteristics seen in diabetic retinopathy.[5] These inspections made VEGF a perceptible goal in the treatment of DME.

Intravitreal anti-VEGF agents like bevacizumab, ranibizumab, and aflibercept, serve as the basis of treatment for DME. Various treatment methods using these agents have been put forward, including monthly, pro re nata, and treat-and-extend regimens.[6] It has been found that response to anti-VEGF alone may be incomplete. Other drugs or combinations are in horizon.

Although the clearance of anti-VEGF agents is not completely perceived, some authors speculate that outflow through the anterior chamber may play a role.[7–9] Sirdhar et al. reported that addition of topical timolol–dorzolamide to fixed-interval anti-VEGF therapy for neovascular age-related macular degeneration (AMD) with continual exudation resulted in a significant reduction in central macular thickness (CMT) and subretinal fluid.[10] Thus, we aimed at evaluating the effectiveness of topical dorzolamide hydrochloride–timolol maleate, as anti-glaucoma agents with aqueous suppressant effects, with intravitreal bevacizumab (IVB) injection in anatomic and visual outcomes in treatment-naive DME patients.

Methods

This prospective study was carried out at Farabi Eye Hospital, during September 2016 to December 2016. Diabetic patients with center-involved macular edema and non-proliferative diabetic retinopathy (non-PDR) were included. Eligible patients were at least 18 years old with type 2 diabetes. All of the patients underwent routine eye examinations, including slit-lamp examination, best corrected visual acuity (BCVA), intraocular pressure (IOP) measurement by the use of Goldmann applanation tonometry, fundus examination, and spectral domain optical coherence tomography (SD-OCT) (Spectralis; Heidelberg Engineering, Heidelberg, Germany) to evaluate the CMT as central subfield thickness. The exclusion criteria were diabetic nephropathy, a history of any ocular and other systemic disorders except diabetes mellitus, a history of any ocular surgery, glaucoma, allergy to beta blockers or carbonic anhydrases compounds, significant cataract which affected patients' vision, epiretinal membrane or vitreoretinal interface abnormalities, proliferative diabetic retinopathy (PDR), and a history of intravitreal injection for treatment DME. Patients with active or regressed PDR or history of retinal photocoagulation, either for DME or PDR were also excluded from the study. Patients were also excluded if their systolic blood pressure was >180 mmHg or diastolic blood pressure was >110 mmHg, or if a myocardial infarction, other cardiac event requiring hospitalization, cerebrovascular accident, transient ischemic attack, or treatment for acute congestive heart failure, or the HbA1c measurement was more than 8.5 or fasting blood sugar (FBS) test was more than 250. Eligible patients were at least 18 years old with type 1 or 2 diabetes. The major eligibility criteria for a study eye included the following: (1) BCVA worse than 20/32, (2) definite retinal thickening due to DME on clinical examination involving the center of the macula assessed to be the main cause of visual loss, and (3) retinal thickness measured on SD-OCT $\geq$300 μm in the central subfield.

Informed written consent was obtained from each participant after explaining the nature of the experimental procedures. The research followed the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Eye Research Center, Tehran University of Medical Sciences.

Three monthly bilateral injections of bevacizumab (Avastin; Genentech, South San Francisco, CA) 1.25 mg/0.05 mL were performed for all patients. The enrolled patients received a therapeutic regimen of topical dorzolamide hydrochloride 2% and timolol maleate 0.5% (Zilomole; Sina Darou, Tehran, Iran) twice per day in the right eye during the study period.

To run the statistical analysis, Snellen acuities were converted to logarithm of the minimum angle of resolution (logMAR) equivalent values. The gleaned data were analyzed with SPSS16 (SPSS, Inc., Chicago, IL). The variables were expressed as mean ± standard deviation (SD). Repeated measures ANOVA test was run to compare the variables within each group. Paired t-test was used to compare the groups ($P < 0.05$).

Results

Eleven treatment-naive patients with the mean age of 54.2 years (range, 46–63 years) were enrolled in the study including seven females and four males. Mean FBS was 218.8, and mean HbA1C was 7.8. In the right eye, which received adjuvant topical timolol–dorzolamide and three monthly IVBs, CMT was reduced (497.63 ± 68.30 vs. 341.27 ± 28.66; $P < 0.001$), BCVA in logMAR scale was reduced (0.52 ± 0.26 vs. 0.19 ± 0.10; $P < 0.001$), and IOP was also reduced (14.36 ± 1.62 vs. 10.72 ± 1; $P < 0.001$). In the left eye, which received three monthly IVBs, CMT was reduced (475.72 ± 82.09 vs. 391.72 ± 45.88; $P < 0.001$), BCVA in logMAR scale was reduced (0.47 ± 0.25 vs. 0.26 ± 0.10; $P = 0.003$), but IOP did not differ significantly (14.81 ± 1.53 vs. 15.45 ± 2.06; $P = 0.172$). Moreover, the changes in CMT (156.36 ± 57.49 vs. 84.00 ± 43.61; $P < 0.001$), BCVA in logMAR scale (0.324 ± 0.213 vs. 0.204 ± 0.175; $P = 0.006$), and IOP (3.63 ± 1.43 vs. −0.63 ± 1.43; $P < 0.001$) in the timolol–dorzolamide treated eyes vs. control eyes were significantly different, respectively. In repeated measures ANOVA analysis between groups, CMT, BCVA in logMAR, and IOP were all reduced significantly in the right eye versus the left eye. The results are summarized in Table 1.

Discussion

Previous reports on topical beta blockers and angiotensin converting enzyme inhibitor have suggested that by reducing

Table 1
Comparison between pre- and post-intravitreal bevacizumab (IVB) in timolol–dorzolamide treated eyes and control contralateral eyes.

	Timolol–dorzolamide treated (right eye)			Control (left eye)			*P* value[a]
	Pre-treatment	Post-treatment	*P* value	Pre-treatment	Post-treatment	*P* value	
Best corrected visual acuity (BCVA) (logMAR)	0.52 ± 0.26	0.19 ± 0.10	<0.001	0.47 ± 0.25	0.26 ± 0.10	0.003	0.007
Central macular thickness (CMT) (microns)	497.63 ± 68.30	341.27 ± 28.66	<0.001	475.72 ± 82.09	391.72 ± 45.88	<0.001	<0.001
Intraocular pressure (IOP) (mmHg)	14.36 ± 1.62	10.72 ± 1	<0.001	14.81 ± 1.53	15.45 ± 2.06	0.172	0.038

[a] Comparison between timolol–dorzolamide treated eye and the control eye.

outflow, anti-VEGF effects may increase.[7,10] Gaudreault et al. showed that ranibizumab concentrations are much lower in the aqueous humor than in the vitreous and it seems to decline in parallel with vitreous levels.[11] Hence, it seems that one of the main routes of elimination of anti-VEGFs may be via aqueous outflow. Byeon et al. showed that in patients with branch or central retinal vein occlusion receiving a single IVB injection for the treatment of macular edema, the mean CMT was reduced in both groups of IVB and IVB with adjuvant timolol–dorzolamide at 1 week after injection, but by 5 weeks, the timolol–dorzolamide group had a lower mean CMT ($P = 0.03$).[7] In a prospective single-arm interventional study on patients with neovascular AMD and persistent macular edema despite fixed-interval intravitreous anti-VEGF therapy, Spridhar et al. found that adjuvant topical timolol–dorzolamide decreases CMT and macular edema and also pigment epithelial detachment height. BCVA improved, but was not significant.[10] These findings suggest that as timolol–dorzolamide has been shown to reduce aqueous flow by approximately 50%, elongation of anti-VEGFs efficiency affects CMT reduction by decreasing outflow.[12,13]

Each component of this combination has also shown effects on retinal vascularization or VEGF. In a mouse model of retinopathy of prematurity, beta blockers reduced upregulation of VEGF and decreased hypoxic retinopathy.[13] In another experimental study, propranolol-treated mice demonstrated a 50% reduction in laser-induced choroidal neovascularization (CNV).[14] In a review based on experimental models, Casini et al. concluded that β2-adrenergic receptor blockade was primarily responsible for the reduced levels of angiogenic factors and retinal neovascularization.[15] Moreover, dorzolamide was effective in the treatment of cystoid macular edema, secondary to postoperative inflammation, retinitis pigmentosa, and also macular changes of X-linked retinoschisis, enhanced S cone disease, and choroideremia.[16–18] Muller cells and retinal pigment epithelial cells were shown to have membrane-bound carbonic anhydrase enzyme.[19] Therefore, dorzolamide may affect Muller cells and retinal pigment epithelial pump function to egress retinal fluid and decrease edema. Dorzolamide was also found to increase retinal and choroidal blood flow.[20] Hence, it cannot be concluded yet that a combination of timolol and dorzolamide, each one by itself, may affect retina and macular edema regardless of application as adjuvant to anti-VEGFs.

In our study, we found that IVB was effective in reducing CMT and improving vision. Adjuvant timolol–dorzolamide improved efficacy of IVB. CMT reduction and BCVA improvement in the eyes that received IVB and timolol–dorzolamide were more prominent. Aqueous outflow reduction was documented as IOP was reduced in the eyes that received timolol–dorzolamide, not in the eyes that received IVB alone. Spridhar et al.[10] have shown that adjuvant timolol–dorzolamide was effective in reducing CMT but was not in improving vision. It was related to chronicity of the macular lesions and atrophy of the outer layers. All the patients were treatment-naive, and diabetic retinopathy was in non-proliferative stage. Improvement of vision in our patients may be related to the nature of the lesions since our patients were diabetic and suffering from CNV. However, avoiding chronicity of the lesion may benefit patients to preserve photoreceptors and outer layers.

It was shown that ranibizumab has been effective in DME as the standard treatment modality. However, bevacizumab is used more frequently for many reasons.[21] Hence, it may be needed to improve the efficacy of bevacizumab. In this study, we have shown that although bevacizumab is effective in improving vision and decreasing the macular thickness, combining topical timolol and dorzolamide improved the efficacy. However, it should be kept in mind that topical modalities were not effective in the treatment of macular edema.[22]

As this study was a contralateral eye study, many confounding factors affecting drug bioavailability were eliminated. On the other hand, our study has many limitations including being a pilot study and a sample size. The duration of the follow-up was also short, and we did not follow the patients till they became edema-free. Moreover, as we used a combinatory product, it seems impossible to distinguish whether the beneficial effect was resulted from a combination of both medications or from each of them alone.

In conclusion, it is suggested that consumption of combination of topical timolol–dorzolamide as adjuvant to anti-VEGFs is effective in the reduction of macular thickness and improvement of vision. Initiating this treatment modality in treatment-naive patients may preserve vision.

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References

1. Zhang X, Zeng H, Bao S, Wang N, Gillies M. Diabetic macular edema: new concepts in patho-physiology and treatment. *Cell Biosci*. 2014;4:27.
2. Ford JA, Elders A, Shyangdan D, Royle P, Waugh N. The relative clinical effectiveness of ranibizumab and bevacizumab in diabetic macular

oedema: an indirect comparison in a systematic review. *BMJ*. 2012;345: e5182.
3. Funatsu H, Yamashita H, Nakamura S, et al. Vitreous levels of pigment epithelium-derived factor and vascular endothelial growth factor are related to diabetic macular edema. *Ophthalmology*. 2006;113(2):294−301.
4. Virgili G, Parragano M, Menchini F, Evans JR. Anti-vascular endothelial growth factor for diabetic macular edema. *Cochrane Database Syst Rev*. 2014;24(10):CD007419.
5. Tolentino MJ, McLeod DS, Taomoto M, Otsuji T, Adamis AP, Lutty GA. Pathologic features of vascular endothelial growth factor-induced retinopathy in the nonhuman primate. *Am J Ophthalmol*. 2002;133(3):373−385.
6. Wykoff CC. Impact of intravitreal pharmacotherapies including anti-vascular endothelial growth factor and corticosteroid agents on diabetic retinopathy. *Curr Opin Ophthalmol*. 2017;28(3):213−218.
7. Byeon SH, Kwon OW, Song JH, Kim SE, Park YS. Prolongation of activity of single intravitreal bevacizumab by adjuvant topical aqueous depressant (timolol-dorzolamide). *Graefes Arch Clin Exp Ophthalmol*. 2009;247(1):35−42.
8. Bakri SJ, Snyder MR, Reid JM, Pulido JS, Singh RJ. Pharmacokinetics of intravitreal bevacizumab (Avastin). *Ophthalmology*. 2007;114(5):855−859.
9. Stewart MW. Predicted biologic activity of intravitreal bevacizumab. *Retina*. 2007;27(9):1196−1200.
10. Sridhar J, Hsu J, Shahlaee A, et al. Topical dorzolamide-timolol with intravitreous anti-vascular endothelial growth factor for neovascular age-related macular degeneration. *JAMA Ophthalmol*. 2016;134(4):437−443.
11. Gaudreault J, Fei D, Rusit J, Suboc P, Shiu V. Preclinical pharmacokinetics of ranibizumab (rhuFabV2) after a single intravitreal administration. *Investig Ophthalmol Vis Sci*. 2005;46(2):726−733.
12. Brubaker RF, Ingram CJ, Schoff EO, Nau CB. Comparison of the efficacy of betaxolol-brinzolamide and timolol-dorzolamide as suppressors of aqueous humor flow in human subjects. *Ophthalmology*. 2000;107(2): 283−287.
13. Ristori C, Filippi L, Dal Monte M, et al. Role of the adrenergic system in a mouse model of oxygen-induced retinopathy: antiangiogenic effects of β-adrenoreceptor blockade. *Investig Ophthalmol Vis Sci*. 2011;52(1): 155−170.
14. Lavine JA, Sang Y, Wang S, Ip MS, Sheibani N. Attenuation of choroidal neovascularization by β2-adrenoreceptor antagonism. *JAMA Ophthalmol*. 2013;131(3):376−382.
15. Casini G, Dal Monte M, Fornaciari I, Filippi L, Bagnoli P. The β-adrenergic system as a possible new target for pharmacologic treatment of neovascular retinal diseases. *Prog Retin Eye Res*. 2014;42:103−129.
16. Thobani A, Fishman GA. The use of carbonic anhydrase inhibitors in the retreatment of cystic macular lesions in retinitis pigmentosa and X-linked retinoschisis. *Retina*. 2011;31(2):312−315.
17. Genead MA, McAnany JJ, Fishman GA. Topical dorzolamide for treatment of cystoid macular edema in patients with choroideremia. *Retina*. 2012;32(4):826−833.
18. Genead MA, Fishman GA, McAnany JJ. Efficacy of topical dorzolamide for treatment of cystic macular lesions in a patient with enhanced S-cone syndrome. *Doc Ophthalmol*. 2010;121(3):231−240.
19. Terashima H, Suzuki K, Kato K, Sugai N. Membrane-bound carbonic anhydrase activity in the rat corneal endothelium and retina. *Jpn J Ophthalmol*. 1996;40(2):142−153.
20. Harris A, Ciulla TA, Pratt LM, et al. The effects of dorzolamide on choroidal and retinal perfusion in non-exudative age related macular degeneration. *Br J Ophthalmol*. 2003;87(6):753−757.
21. Miller CG, Budoff G, Jeng-Miller KW, Fine HF, Roth DB, Prenner JL. Retina specialists treating diabetic macular edema recommend different approaches for patients than they would choose for themselves. *Ophthalmic Surg Lasers Imaging Retin*. 2016;47(6):544−554.
22. Friedman SM, Almukhtar TH, Baker CW, et al. Diabetic retinopathy clinical research network. Topical nepafenac in eyes with noncentral diabetic macular edema. *Retina*. 2015;35(5):944−956.