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ORIGINAL RESEARCH

Comparative Cross-sectional Analysis of the Effects of Topical Antiglaucoma Drugs on the Ocular Surface

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ABSTRACT

Introduction: The aim of this study was to comparatively analyze the effects of topical intraocular pressure (IOP)-lowering drugs on the ocular surface and to elucidate whether the main causative factor of toxicity is associated with benzalkonium chloride (BAK) or an active compound.

Methods: The medical records of 300 eyes in 187 glaucoma patients that had instilled IOP-lowering drugs were cross-sectionally reviewed. Corneal epithelial punctuate erosion and tear break-up time (BUT) were quantitatively assessed. Durations of glaucoma, sums of concentrations of BAK in current medication ($BAK_{\%sum}$), and the presence of beta-blockers were investigated as risk factors (Institutional Review Board of Seoul National University Hospital, Seoul – IRB number: H-1007-103-324).

Results: Age-adjusted $BAK_{\%sum}$ was found to be significantly and positively correlated with corneal epithelial punctate erosion ($P = 0.001$, $r = 0.208$) and negatively correlated with BUT ($P = 0.042$, $r = 0.131$). $BAK_{\%sum}$ adjusted corneal epithelial erosion was found to be significantly greater in beta-blocker containing eyedrop-instilled eyes ($P = 0.016$). No difference in ocular toxicity was found between carbonic anhydrase inhibitor and prostaglandin analog or between latanoprost- and travoprost-treated eyes.

Conclusion: Long-term treatment with BAK-containing antiglaucoma medication appears

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to be the main contributor to corneal toxicity and to do so in a dose-dependent manner. Formulations containing beta-blockers also appear to contribute to corneal toxicity.

Keywords: Benzalkonium chloride; Beta-blocker; Cornea; Epithelial erosion; Glaucoma; Toxicity

INTRODUCTION

The prevalence of glaucoma is 1.9–2.1% in patients >40 years old, and the condition is more common in older individuals [1, 2]. Topical intraocular pressure (IOP)-lowering drugs are a mainstay of treatment for glaucoma in almost all cases these days, and most patients require life-long topical drop treatment after diagnosis. Some patients subsequently suffer from corneal surface problems during treatment [3–6].

The majority of IOP-lowering drugs contain preservative, and long-term treatment with preservative-containing eyedrops is known to cause or worsen ocular surface disease (OSD) [3–6]. Benzalkonium chloride (BAK) is the most popular preservative used and is considered to have a harmful effect on the ocular surface [7, 8]. Corneal toxicity remains of primary concern in patients on long-term BAK-containing antiglaucoma eyedrop treatment [9, 10].

Recent studies have addressed relations between subjective changes in the ocular surface using the OSD index (OSDI) and a number of glaucoma medications, or have examined the correlations between the presence of corneal erosion, break-up time (BUT), or Schirmer's test outcome and the number of glaucoma medications [9, 10]. However, these studies presented semi-quantitative, nonspecific evidence regarding the effect of antiglaucoma medication on the ocular surface [11], and no study has demonstrated quantitatively the dose-dependent effect of BAK in antiglaucoma medication on corneal epithelial damage in people. In addition, other factors, such as active ingredients or solution formulae, could also cause effect on the ocular surface, and no comparative investigation has been undertaken to examine relations between the effects of different formulas on corneal epithelial damage. Thus, it is uncertain whether corneal toxicity is due to the deleterious effect of BAK or to the cumulative toxicity of various active compounds.

It was hypothesized that BAK or antiglaucoma formula-associated corneal toxicity causes corneal toxicity dose-dependently. Accordingly, a comparative analysis was carried out on the cumulative effect of BAK in antiglaucoma medication and the formula-associated effects of different antiglaucoma drops on the corneal surface.

METHODS

This cross-sectional study was carried out with the approval of the Institutional Review Board of Seoul National University Hospital, Seoul (IRB number: H-1007-103-324). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

Patients with a diagnosis of glaucoma and regularly followed-up in a glaucoma clinic (D. M. Kim) at Seoul National University Hospital were recruited from May to August 2010. Corneal surfaces were cross-sectionally assessed once in each patient during this period.

In total, 300 eyes were enrolled in this study, which was presumed to be sufficient to estimate correlation between beta-blocker and corneal toxicity based on a power calculation.

Inclusion criteria for the study included: (1) 20–80 years of age; (2) a clinical diagnosis of normal tension glaucoma, primary open-angle glaucoma, chronic angle-closure glaucoma, steroid-induced glaucoma, or suspected glaucoma; (3) the instillation of the same IOP-lowering drugs for at least 8 weeks without any drug change; and (4) the provision of signed informed consent. Exclusion criteria included: (1) a clinical diagnosis of uveitic glaucoma; (2) a history of herpes keratitis confirmed by a positive polymerase chain reaction finding; (3) another OSD, such as, Stevens-Johnson syndrome, rosacea, Sjögren's syndrome, or a history of dry eye syndrome; (4) a history of ocular surgery, except cataract surgery >3 months previously; (5) diabetes mellitus; (6) current contact lens use; (7) current use or use within the previous 3 months of Restasis® (Allergan, Inc., Irvine, CA, USA), steroids, or topical nonsteroidal anti-inflammatory drugs; and (8) poor adherence to medication during the enrollment.

In each patient, the corneal surface was blindly assessed by examining the extent of corneal epithelial punctate erosion and by measuring tear BUT. Examinations were performed using a slit lamp at 10–16 x magnification using cobalt illumination after instilling a drop of 0.25% fluorescein solution. Corneal epithelial punctate erosion was evaluated by area and density. Densities were recorded as D0, D1, D2, or D3, and areas as A0, A1, A2, and A3, as previously described (Table 1) [12]. Corneal epithelial erosion scores were calculated by multiplying D and A scores. The time of testing tear BUT was measured for up to 10 seconds.

To evaluate the risk factors of corneal toxicity in a quantitative manner, duration of glaucoma and cumulative daily dosages of BAK were measured. Duration of glaucoma was defined as time from commencing the instillation of any type of IOP-lowering drugs to control glaucoma, and it was recorded in months. The concentrations of BAK of each instilling drug were multiplied by daily instillation frequencies a day and defined as $BAK_{\%sum}$. Correlations between duration of glaucoma or $BAK_{\%sum}$ and corneal epithelial punctate erosion or BUT were assessed by linear regression analysis and multivariate analysis (adjusted for age).

Active compounds (i.e., beta-blocker, prostaglandin), as well as preservatives, may affect the ocular surface. To determine whether active compounds in beta-blocker eyedrops are involved in corneal toxicity, subjects were divided into two sets of two groups based on the presence of beta-blocker and corneal toxicity and compared. The eyes being instilled with IOP-lowering drugs containing a beta-blocker, including fixed-combination drugs (e.g., dorzolamide and timolol maleate fixed combination [D-TM; Cosopt®, Merck & Co. Inc.,

Table 1 Classification of corneal epithelial lesion severity

Area : corneal surface area	Density : density of damaged lesions
A0 : No punctuate staining	D0 : No punctuate staining
A1 : <1/3	D1 : Mild density
A2 : 1/3 ~ 2/3	D2 : Moderate density
A3 : >2/3	D3 : High density with overlapping lesions

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Whitehouse Station, NJ, USA], timolol maleate [Timabak®, Laboratoires Théa, Clermont-Ferrand, France], or latanoprost and timolol maleate fixed combination [L-TM; Xalacom®, Pfizer Inc., New York, NY, USA]), were enrolled in the beta-blocker group (n = 185) and those that applied non-beta-blockers, e.g., travoprost (Travatan®, Alcon Laboratories Inc., Fort Worth, TX, USA), dorzolamide (Trusopt®, Merck & Co. Inc., Whitehouse Station, NJ, USA), or latanoprost (Xalatan®, Pfizer Inc., New York, NY, USA) were assigned to the non-beta-blocker group (n = 115). For a full list of drugs included in this study see Table 2. Statistical significance versus the non-beta-blocker group was determined by multiple regression analysis. To perform the analysis, the beta-blocker and non-beta-blocker groups were converted into dummy variables (1 and 0, respectively) and $BAK_{\%sum}$ was set as a covariate.

To determine whether different formulas affect corneal toxicity, patients were divided into subgroups based on topical medication as follows; latanoprost versus L-TM (n = 47, 17); dorzolamide versus D-TM (n = 9, 30); latanoprost versus travoprost (n = 47, 7); latanoprost versus dorzolamide (n = 47, 9); dorzolamide versus travoprost (n = 9, 7). Corneal erosion scores and BUT values of subgroups were compared by multivariate analysis adjusted for BAK concentration. To perform this analysis, subgroups were also converted into dummy variables of 1 and 0, and the concentration of BAK in each topical medication was set as a covariate. BAK adjustment was not performed when BAK concentrations in drugs were equal.

The characteristics of the topical glaucoma drops addressed in this study are summarized in Table 2. Latanoprost and L-TM had the same concentration of latanoprost and BAK; in addition, L-TM contains 0.5% of timolol maleate. The pH values and osmolalities of latanoprost and L-TM are 6.7 and 6.0, and 267 and 290 mOsmol/kg, respectively. Dorzolamide and D-TM have the same dorzolamide and BAK concentration, and D-TM also contains 0.5% timolol maleate. The pH values of dorzolamide and D-TM are 5.6 and 5.65, and their osmolalities are 260–330 and 242–323 mOsmol/kg, respectively, and travoprost contains 0.004% of travoprost and its pH and osmolality are 6.0 and 290 mOsmol/kg, respectively.

Statistical analysis was performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA), and statistical significance was accepted for $P < 0.05$. The independent Student's t-test for comparison of mean values, Spearman correlation for correlation analysis, and multiple linear regression model for multivariate analysis were used.

RESULTS

Demographic data

In total, 300 eyes of 187 patients were included, and 142 eyes of 90 patients were male. Mean ages of males and females were 59.5 ± 15.0 and 58.5 ± 14.3 years, respectively, and there was no significant difference in mean ages between genders (P = 0.637; Table 3). Eighty-four (28.0%) eyes had corneal epithelial punctate erosion (Table 4) and most of these eyes had mild erosion with a presenting score of <3, and 194 (64.7%) eyes had a BUT of <10 s.

Gender

Women had denser corneal epithelial punctate erosion (P = 0.015, Student's t-test) but corneal erosion scores were not significantly different between genders (P = 0.233). BUT was significantly shorter in female patients (P = 0.019), but $BAK_{\%sum}$ was not significantly different between genders (P = 0.397) (Table 3).

Table 2 The characteristics of the topical glaucoma drops administered in this study were shown

	Active compound	Preservative	pH	Osmolality (mOsm/kg)	Dosage
Single drug					
Alpha-agonist	Apraclonidine 1%	BAK 0.01%	4.4–7.8	260–320	b.i.d.
	Brimonidine 0.15%	PURITE® 0.005%	6.9–7.4	250–350	b.i.d.
Beta-blocker	Betaxolol 0.25%	BAK 0.01%	7.60	290	b.i.d.
	Calteolol 2%	BAK 0.005%	NA	NA	b.i.d.
	Calteolol 2%	BAK 0.005%	6.2–7.2	NA	q.d.
	Nipradilol 0.25%	BAK 0.01%	NA	NA	b.i.d.
	Timolol 0.5%	BAK 0.001%	7.2–8.0	NA	q.d.
	Timolol 0.5%	None	6.90	294	b.i.d.
	Timolol 0.5%	Benzododecinium bromide 0.012%	7.00	260–330	q.d.
CAI	Brinzolamide 1%	BAK 0.01%	7.50	300	b.i.d.
	Dorzolamide 2%	BAK 0.0075%	5.60	260–330	b.i.d.
PG	Bimatoprost 0.03%	BAK 0.005%	6.8–7.8	290	q.d.
	Latanoprost 0.005%	BAK 0.02%	6.70	267	q.d.
	Travoprost 0.004%	BAK 0.015%	6.00	290	q.d.
Fixed combination drug with 0.5% timolol					
Alpha-agonist	Brimonidine 0.2%	BAK 0.005%	6.5–7.3	260–330	b.i.d.
CAI	Brinzolamide 1%	BAK 0.01%	7.20	NA	b.i.d.
	Dorzolamide 2%	BAK 0.0075%	5.65	242–323	b.i.d.
PG	Bimatoprost 0.03%	BAK 0.005%	7.2–7.4	270 –310	q.d.
	Latanoprost 0.005%	BAK 0.02%	6.00	290	q.d.
	Travoprost 0.004%	BAK 0.015%	6.00	290	q.d.

BAK benzalkonium chloride, *b.i.d.* twice daily, *CAI* carbonic anhydrase inhibitor, *NA* information not available, *PG* prostaglandin analog, *q.d.* four times daily

Table 3 Ocular surface disease parameters by gender

Parameter	Male (n = 142)	Female (n = 158)	P value[a]
Area	0.34 ± 0.73	0.44 ± 0.72	0.212
Density	0.34 ± 0.70	0.57 ± 0.92	0.015
Area x density	0.55 ± 1.50	0.74 ± 1.27	0.233
BUT (second)	7.50 ± 2.25	6.83 ± 2.34	0.019
$BAK_{\%sum}$ (%)	0.022 ± 0.011	0.021 ± 0.010	0.397
Duration of glaucoma (month)	74.8 ± 58.0	61.9 ± 55.3	0.050

$BAK_{\%sum}$ benzalkonium chloride in current medication, *BUT* break-up time
[a] Independent Student's t-test

Table 4 Semi-quantitative analysis of punctuate corneal erosion[a] in enrolled patients

Corneal erosion	Corneal erosion score	Number of eyes (n = 300)	Total
Clear	0	216 (72.0%)	216 (72.0%)
Mild	1	29 (9.7%)	60 (20.0%)
	2	31 (10.3%)	
Moderate	3	13 (4.3%)	18 (6.0%)
	4	5 (1.7%)	
Severe	6	3 (1.0%)	6 (2.0%)
	9	3 (1.0%)	

[a] Calculated by multiplying involved area by erosion density

Duration of Glaucoma

Age-adjusted duration of glaucoma was not found to be correlated with corneal epithelial punctate erosion (P = 0.973, r = 0.075) or BUT (P = 0.728, r = 0.043).

$BAK_{\%sum}$

Age-adjusted $BAK_{\%sum}$ was significantly and positively correlated with corneal epithelial punctate erosion (P = 0.001, r = 0.208, Spearman correlation) and negatively correlated with BUT (P = 0.042, r = –0.131, Spearman correlation) (Fig. 1).

Presence of Beta-Blockers

Eyes being instilled with any type of beta-blocker (four different kinds of beta-blockers were included; timolol maleate, betaxolol, calteolol, and nipradilol; Table 2) had more corneal epithelial punctate erosion (beta-blocker [–] group : beta-blocker [+] group = 0.35 ± 0.77 : 0.84 ± 1.63, P = 0.001) and a shorter BUT (beta-blocker [–] group : beta-blocker [+] group = 7.53 ± 2.39 : 6.89 ± 2.24, P = 0.027). $BAK_{\%sum}$-adjusted corneal epithelial punctate erosion was significantly severer in the beta-blocker (+) group (P = 0.016), but $BAK_{\%sum}$-adjusted BUT was not significantly different between beta-blocker (+) and (–) groups (P = 0.063) (Fig. 2).

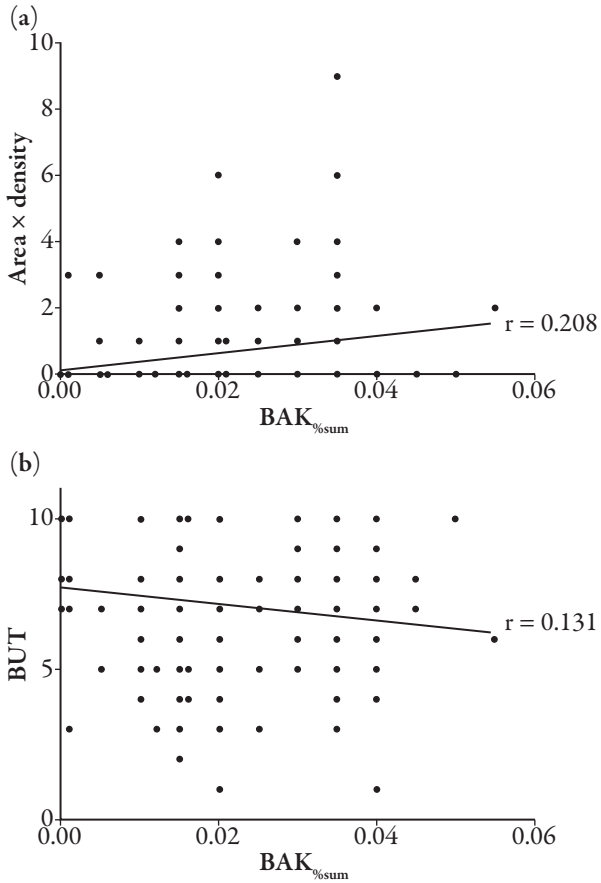

Fig. 1 Scatter plots showing a positive correlation between $BAK_{\%sum}$ and corneal epithelial punctate erosion in total enrolled patients (**a**) and a negative correlation between $BAK_{\%sum}$ and BUT (**b**). *$BAK_{\%sum}$* benzalkonium chloride in current medication, *BUT* break-up time

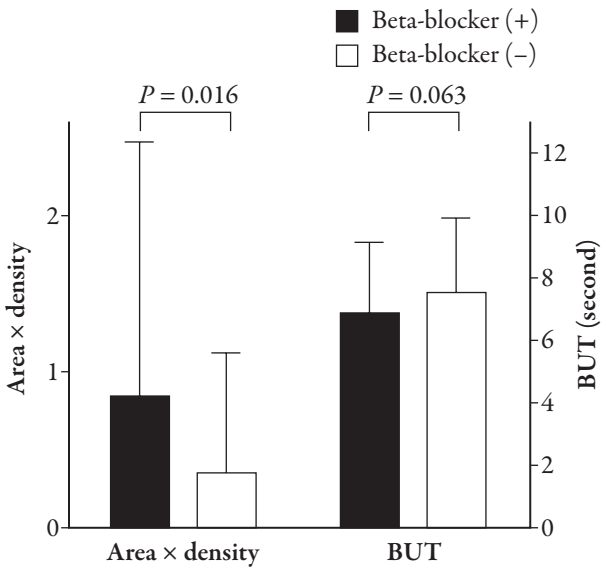

Fig. 2 $BAK_{\%sum}$-adjusted corneal epithelial punctate erosion was significantly more severe in the beta-blocker instilling group, but $BAK_{\%sum}$-adjusted BUT was not significantly different between the beta-blocker (+) and (–) groups (multivariate analysis). *$BAK_{\%sum}$* benzalkonium chloride in current medication, *BUT* break-up time

Comparison of Different Formulae After Adjusting for BAK Concentrations

There were no differences in BUT and corneal erosion between subgroups: latanoprost versus travoprost, latanoprost versus dorzolamide, travoprost versus dorzolamide, latanoprost versus L-TM, and dorzolamide versus D-TM (data not shown).

DISCUSSION

The present study shows that BAK is a primary contributor to the corneal toxicity of antiglaucoma medications, and that beta-blocker formulation possibly contributes to corneal toxicity. No clinically relevant difference was found between the corneal surface toxicities of carbonic anhydrase inhibitor and treatments containing prostaglandin analog.

The treatment of glaucoma generally requires life-long topical medication, therefore glaucoma patients exhibit a high prevalence of OSD [9, 10, 13]. Given glaucoma is more common in the elderly, glaucoma patients may also be at higher risk of developing OSD than the overall population [10]. Several plausible factors could cause the corneal toxicity of topical glaucoma medications; they are, preservatives, a lower or higher pH than neutral in conjunctiva, a non-isotonic osmolality, and active compounds. Furthermore, many reports have suggested that preservatives are the main reason for OSD induction by topical antiglaucoma medications [7, 8], and other experimental reports have demonstrated BAK can cause corneal epithelial toxicity [14, 15]. This study supports previous suggestions that BAK is a major contributory factor.

Recent clinical studies that have investigated the association between corneal toxicity and antiglaucoma drops have found it difficult to account for many formulation-related variables, for example, many different kinds of drops, based on different formulas, were used by individual patients over time [10]. In addition, previous studies lacked objective, quantitative measures of corneal toxicity [9, 16]. In fact, although a number of topical antiglaucoma medications, presence of corneal erosion, BUT, and OSDI have been used, subsequent analyses crudely looked into relationships between numbers of topical medications and those indices mentioned above [9, 10]. In the present study, the authors adopted a strict objective, quantitative approach by using an objective measure of corneal erosion and BAK dosage.

Several adverse effects on the ocular surface have been reported for topically applied active compounds, such as timolol maleate or prostaglandins. Usage of topical timolol maleate has been associated anecdotally with

conjunctival hyperemia, superficial punctate keratitis, dry eye, allergic blepharoconjunctivitis, reduced BUT and Schirmer's test results, nonuniformity of the tear lipid layer, and reduced corneal sensitivity causing corneal anesthetic effect [7, 12, 17–22]. This corneal anesthetic effect of timolol maleate may have attributed to significant corneal toxicity in beta-blocker instilling patients which was presented in this study [23, 24].

The application of latanoprost has been associated with superficial punctate keratitis, and herpes simplex dendritic keratitis [17–19, 25, 26], and the administration of dorzolamide with superficial punctate keratitis, corneal decompensation, and increased central corneal thickness [27, 28]. However, it has not been determined whether these effects are caused by active compounds or by a combination of active compounds and preservative. In the present study, multivariate analysis showed that corneal epithelial punctate erosion was more severe in the beta-blocker group ($P = 0.016$) than in the non-beta-blocker group. The present study indicates that the presence of a beta-blocker is associated with the development of OSD, which in turn suggests that not only preservatives but also active compound per se should be considered a toxic factor. However, due to the size of the cohort comparisons of BAK-free timolol and the other timolol-containing drugs were not possible, to exclude the effect of BAK. Nevertheless, the authors have often encountered corneal erosion or conjunctival hyperemia in patients that have applied BAK-free timolol, which resulted in the examination of the corneal toxicity of the timolol maleate. A prospective, randomized, case-controlled study should be conducted to clarify this issue.

It was also interesting to find no clinical difference between the ocular toxicities of carbonic anhydrase and prostaglandin analog or between latanoprost and travoprost, which provides clinically relevant clues regarding changes in medication when corneal toxicity in encountered. It is the suggestion of the authors that BAK-free or near BAK-free topical drops should be considered initially in this circumstance rather than changing to drops containing a different active compound.

Although the analyses of single drugs and fixed combinations containing a beta-blocker failed to show that timolol maleate has a detrimental effect, it has been shown in another study that timolol maleate is linked to OSD [29].

This study has several limitations. First, in addition to BAK and active compounds, other additives, pH, and osmolality might have effected OSD, but it was not possible to account for all these confounders in the analyses. Second, recent studies have shown the shortage of utility of BUT in the detection of OSD, particularly in mild/moderate dry eye [30, 31]. Tear osmolarity can be an objective tool in the evaluation of dry eye but it was not measured in this study [30, 31]. Third, there might be some bias, as more affected individuals who had used eyedrops containing either BAK or a beta-blocker may change their medications following physician's prescription. It can affect to attenuate the correlation in scatterplot despite the adverse effect of BAK and the beta-blocker may be even stronger than reported since the treating physician may change treatment plans for patients with significant OSD. Fourth, the cross-sectional design adopted was inherently limited in terms of determining the nature of the dose-response relationship, because the duration and the initiation of being medicated by glaucoma-drop were selected. And most decisively, there was no control group with patients who were instilling BAK-free eyedrops. Thus, the deleterious effect of BAK could not be strongly supported by the

data shown here. A longitudinal prospective study is required to clarify this issue, which was overcome in part by calculating BAK concentrations and adjusting for some variables such as age and the final concentration of BAK during the multivariate analysis. This would provide meaningful findings as well as providing supportive evidence with quantitative manner on top of previous reports.

CONCLUSION

The present study shows BAK, in topical medication, dose-dependently increases the risk of OSD development in glaucoma patients, and that the presence of a beta-blocker as an active compound seemed to contribute to this risk. The findings suggest that BAK-containing IOP-lowering drugs should be replaced by a BAK-free IOP-lowering drug in patients with OSD, and that formulations containing a beta-blocker should be discontinued in patients with OSD.

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Conflict of Interest. Dr. Sangmoon Lee, Dr. Mee Kum Kim, Dr. Hyuk Jin Choi, Dr. Won Ryang Wee, and Dr. Dong Myung Kim declare no conflict of interest.

Compliance with Ethics Guidelines. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

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