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Effect of Combination Use of Aqueous Humor Secretion Inhibitor Eye Drops on Aflibercept Level: A Preliminary Analysis

Satoru Inoda[1], Hidenori Takahashi[1], Ryota Takahashi[1], Yuto Hashimoto[1], Hana Yoshida[1], Hironori Takahashi[1], Yujiro Fujino[2], Kenichi Aizawa[3,4], Hidetoshi Kawashima[1], and Yasuo Yanagi[5,6]

[1] Department of Ophthalmology, Jichi Medical University, Shimotsuke-shi, Tochigi, Japan
[2] Department of Ophthalmology, Japan Community Healthcare Organization Tokyo Shinjuku Medical Center, Tokyo, Japan
[3] Division of Clinical Pharmacology, Department of Pharmacology, Jichi Medical University, Tochigi, Japan
[4] Clinical Pharmacology Center, Jichi Medical University Hospital, Tochigi, Japan
[5] Department of Ophthalmology and Micro-Technology, Yokohama City University, Yokohama, Japan
[6] Retina Research Group, Singapore Eye Research Institute, Singapore Eye-ACP, Duke-NUS Medical School, National University of Singapore, Singapore

Correspondence: Hidenori Takahashi, Department of Ophthalmology, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke-shi, Tochigi 329-0431, Japan. e-mail: takahah-tky@umin.ac.jp

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Purpose: To investigate the association between aqueous humor (AH) suppressant eye drops and the concentration of aflibercept at 1 month after intravitreal injection.

Methods: This retrospective study included 17 eyes of 17 patients with neovascular age-related macular degeneration (nAMD) who used eye drops for their glaucoma and received their first intravitreal aflibercept (IVA) at two centers between July 2013 and November 2020. As controls, we enrolled 40 age-, sex-, and axial length–matched eyes of 40 patients with nAMD who were not using any medication that would affect AH circulation. AH was collected 1 month after the first IVA. Aflibercept levels were measured by enzyme-linked immunosorbent assay and were compared between controls and cases using the Kruskal–Wallis test and Dunn's test. The drugs were categorized into two groups based on their mechanism of action on the AH: outflow drugs (e.g., prostaglandin analog) and inflow drugs (e.g., carbonic anhydrase inhibitor, beta-blockers, and alpha-2 agonists).

Results: Mean (interquartile range) aflibercept levels in the AH in controls and in cases who used outflow and inflow drugs were 6.83 µg/mL (1.94–10.34), 9.93 µg/mL (2.58–17.44), and 15.95 µg/mL (7.20–22.57), respectively. A Kruskal–Wallis test showed a significant difference among the control, inflow, and outflow drugs ($P = 0.0075$). Dunn's test showed that aflibercept levels in the aqueous humor were significantly higher in cases using inflow drugs compared to both controls and cases using outflow drugs ($P = 0.0085$ and $P = 0.044$, respectively).

Conclusions: Aflibercept levels in the AH 1 month after the first IVA were higher in cases using eye drops that reduce AH secretion than in controls.

Translational Relevance: Our results, together with previous studies in animals, suggest that combined use of these eye drops might extend the half-life of intravitreally injected drugs.

Introduction

Since their introduction, anti–vascular endothelial growth factor (VEGF) agents have shown proven effectiveness in the treatment of neovascular age-related macular degeneration (nAMD). The standard of care is generally the treat-and-extend regimen, which maintains efficacy while reducing burden. Despite the favorable effects of anti-VEGF agents, anti-VEGF therapy entails considerable patient burden due to the need for regular intravitreal injections. Newly launched anti-VEGF agents have been designed and developed with the aim of extending their half-life and reducing the frequency of injections. A small VEGF-binding molecule such as brolucizumab (26 kDa) allows for a higher clinical dose in the same volume that can be safely injected intravitreally, potentially prolonging the clinical activity compared to larger molecules such as bevacizumab (149 kDa).[1] The TENAYA and LUCERNE studies showed that the effect of faricimab, which targets not only VEGF but also angiopoietin 2, appears to be prolonged compared to aflibercept in terms of maintaining best-corrected visual acuity.[2]

The pharmacokinetics of anti-VEGF agents have been studied using a variety of approaches, including in vivo experiments in rabbits following either intravenous or intravitreal injections. Anti-VEGF agents injected into the eye are not normally metabolized or degraded but are primarily cleared from the vitreous cavity by the anterior pathway.[3–7] This means that anti-VEGF drugs passively diffuse into the aqueous humor and are released into the systemic circulation. The circulation of the aqueous humor is controlled by the balance between its secretion and reabsorption, with reabsorption being a passive process. Among eye drops, those for glaucoma lower the intraocular pressure by affecting aqueous humor circulation, and some in particular inhibit aqueous humor secretion and circulation. For example, carbonic anhydrase inhibitors (CAIs), beta-blockers, and alpha-2 agonists are widely used to lower intraocular pressure by reducing aqueous humor secretion, and prostaglandin analogs (PGAs) and alpha-2 agonists are also used to reduce resistance to aqueous humor outflow.[8,9]

The purpose of this study was to test our hypothesis that, given that anti-VEGF drugs are passively cleared from the eye, slower aqueous humor turnover could potentially lead to delayed clearance of anti-VEGF drugs, thereby prolonging their half-life.

Methods

Ethical Approval and Consent

This case–control study had a two-center, retrospective design. The protocol was approved by the institutional review boards of Jichi Medical University (JICHI20-127) and the Japan Community Healthcare Organization Tokyo Shinjuku Medical Center (CU20-R007, H22-3) and adhered to the tenets of the Declaration of Helsinki and its later amendments or comparable ethical standards. The study procedures followed our institutional guidelines, and all patients provided oral informed consent before the procedures were performed.

Procedure

This retrospective study included 17 eyes of 17 patients with nAMD who used eye drops for glaucoma and were treated with the first aflibercept 2-mg injection at one of the two centers between July 2013 and November 2020. As controls, we used 40 age-matched eyes of 40 patients with nAMD who did not use any eye drops or medication that would affect aqueous humor circulation and who were also treated with the first aflibercept 2-mg injection. We collected ∼0.2 mL of aqueous humor into a disposable syringe just prior to the second aflibercept injection. The liquid was immediately transferred to a sterile tube (ProteoSave; Sumitomo Bakelite Co., Ltd., Tokyo, Japan) and stored at −80°C until measurement. Aqueous humor samples used for the measurements were taken 1 month after the first injection. Aflibercept concentrations were measured using the Aflibercept ELISA Kit (IG-AA115; AybayTech Biotechnology Company, Ankara, Turkey).

Cases and Controls

Inclusion criteria for cases were as follows: nAMD patients with glaucoma who received the first aflibercept injection without other ocular diseases and who had no prior history of any glaucoma surgery. Controls had nAMD without other ocular diseases. For the control group, we considered the baseline clinical characteristics (age, sex, axial length, time from aflibercept injection to aqueous humor sampling, and presence or absence of vitreous) as potential confounders responsible for selection bias. Participants were included if the patient received an aflibercept injection between 28 and 35 days before the aqueous humor sampling.

Then, from 316 patients (median age, 75 years; 69% male; median axial length, 23.4 mm), we excluded patients who had undergone vitrectomy. Next, we selected 17 cases at approximately one case for two controls. These cases were matched for age (±1 year), sex, and axial length (±1 mm).

Among cases, 12 eyes had normal-tension glaucoma, four had primary open-angle glaucoma, and one had secondary glaucoma due to sarcoidosis. Ten eyes had type 1 macular neovascularization (MNV), one eye had type 2 MNV, and six eyes had polypoidal choroidal vasculopathy. The cases used PGAs (bimatoprost, travoprost, latanoprost, isopropyl unoprostone, and tafluprost), beta-blockers (carteolol hydrochloride and timolol maleate), CAIs (brinzolamide and dorzolamide hydrochloride), and/or an alpha-2 agonist (brimonidine tartrate). Among the 17 cases, 10 patients used a single eyedrop. Of these 10 patients, seven used a PGA (three, tafluprost; two, latanoprost; one travoprost; one, isopropyl unoprostone) and the remaining three used a CAI (dorzolamide hydrochloride), beta-blocker (carteolol hydrochloride), or alpha-2 agonist (brimonidine tartrate). Among the other seven patients, two used a PGA and a beta-blocker (tafluprost/timolol fixed-dose or travoprost/timolol fixed-dose); two used a PGA and an alpha-2 agonist (latanoprost and brimonidine tartrate); one used a CAI and a beta-blocker (brinzolamide/timolol fixed-dose) and an alpha-2 agonist (brimonidine tartrate); one used a CAI and a beta-blocker (brinzolamide/timolol fixed-dose) and a PGA (bimatoprost); and one used a CAI and a beta-blocker (dorzolamide hydrochloride/timolol), an alpha-2 agonist (brimonidine tartrate), and a PGA (bimatoprost). Among the 40 controls, 12 had type 1 MNV, two had type 2 MNV, three had type 3 MNV, and 23 had polypoidal choroidal vasculopathy.

Statistical Analysis

Cases were classified as PGA eyedrop users, beta-blocker eyedrop users, CAI eyedrop users, and alpha-2 agonist eyedrop users. Seven patients used a PGA alone, and one patient each used a CAI, beta-blocker, or alpha-2 agonist alone. To evaluate the effect of eye drops that inhibit aqueous humor secretion on pharmacokinetics after intravitreal injection, cases who used more than one type of eye drop were analyzed in duplicate as users of either type. The drugs were categorized into two groups based on their mechanism of action on the aqueous humor: outflow drugs (e.g., PGA) and inflow drugs (e.g., CAI, beta-blockers, alpha-2 agonists). For the purpose of this analysis, we sought to isolate drugs that exhibited purely outflow effects and classified them as "outflow drugs." Therefore, alpha-2 agonists, which possess additional pharmacological actions beyond outflow effects, were not included in this category. Thus, for example, a case who used prostaglandin, beta-blocker, and CAI eye drops was analyzed as an outflow drug user and as an inflow drug user. Aflibercept levels were compared between controls and cases using the Kruskal–Wallis test and Dunn's test. To further clarify the association between aflibercept levels and the type of eye drop used, multivariate analysis was conducted, including parameters such as age, sex, axial length, and time from aflibercept injection to aqueous humor sampling. To address the small sample size, we performed multivariate analyses using two models with a reduced set of independent variables. Categorical data were assessed using Pearson's χ^2 test or Fisher's direct probability test. All statistical analyses were performed using JMP Pro 17.0.0 software (SAS Institute, Cary, NC). $P < 0.05$ was considered statistically significant. Results are reported as mean (interquartile range [IQR]).

Predicted Biological Activity

A previous report showed that the biological activity of VEGF-Trap was maintained for 79 to 87 days.[10] Based on this result, we set the biological activity of VEGF-Trap at an average of 84 days. Peak intravitreal aflibercept concentrations were observed approximately 1 day after intravitreal injection.[11] These concentrations were calculated using the 28- and 84-day intravitreal aflibercept concentrations in the control group as follows:

$$A_1 = A_{28} \times 2^{\frac{A_{28}-A_1}{T_{1/2}}}$$

$$A_{84} = 28\,d \cdot \left(\frac{1}{2}\right)^{\frac{84\,\text{days}-28\,\text{days}}{T_{1/2}}}$$

where A_1 is the aflibercept level in the aqueous humor one day after aflibercept injection (μg/mL); A_{28} is the aflibercept level in the aqueous humor 28 days after aflibercept injection (μg/mL); and $T_{1/2}$ is the half-life time of aflibercept.

The half-life, when combined with eye drops, is calculated based on the intravitreal aflibercept concentration measured 28 days post-injection as follows:

$$T_{d1/2} = \frac{28\,\text{days} - 1\,\text{day}}{\log_2 \frac{A_{d1}}{A_{d28}}}$$

where $T_{d1/2}$ is the half-life of aflibercept in combination with topical eye drops; A_{d1} is the aflibercept level in the aqueous humor 1 day after aflibercept injection in

combination with topical eye drops (µg/mL); and A_{d28} is the aflibercept level in the aqueous humor 28 days after aflibercept injection in combination with topical eye drops (µg/mL).

By utilizing the half-life ($T_{d1/2}$) when eye drops were used, we calculated the time taken for aflibercept concentration to reach the effective inhibitory concentration, which is equivalent to the concentration in the control group at 84 days post-injection (A_{84}), when combined with eye drops:

$$\text{Days} = \log_2 \frac{A_{28}}{A_{84}} \cdot T_{d12}$$

Results

Patient Demographics

Patient characteristics are shown in Table 1. The mean (IQR) ages of the controls and cases were 73.6 (70.3–78.0) years and 74.1 (69.0–79.0) years, respectively. The mean (IQR) times from aflibercept injection to aqueous humor sampling in the controls and cases were 29.9 (29.0–30.8) days and 29.2 (27.9–30.6) days, respectively. There was no significant difference in intraocular pressure between controls and cases ($P = 0.75$). All patients had consistently used glaucoma eye drops for at least 1 year.

The characteristics of controls and cases by eye drops used are shown in Supplementary Table S1. There was no significant difference in the time from aflibercept injection to aqueous humor sampling between controls and cases ($P = 0.18$) or specifically among cases using a CAI, beta-blocker, or alpha-2 agonist ($P = 0.60$, $P = 0.60$, and $P = 0.80$, respectively), but the time was significantly shorter in cases who used a PGA ($P = 0.021$). Although no outliers were identified, the distributions of the data differed, suggesting a statistically significant difference. Axial length was not significantly different between controls and cases ($P = 0.52$) or specifically among cases who used any eye drop ($P = 0.71$, $P = 0.70$, $P = 0.71$, and $P = 0.49$, for PGA, CAI, beta-blocker, and alpha-2 agonist, respectively).

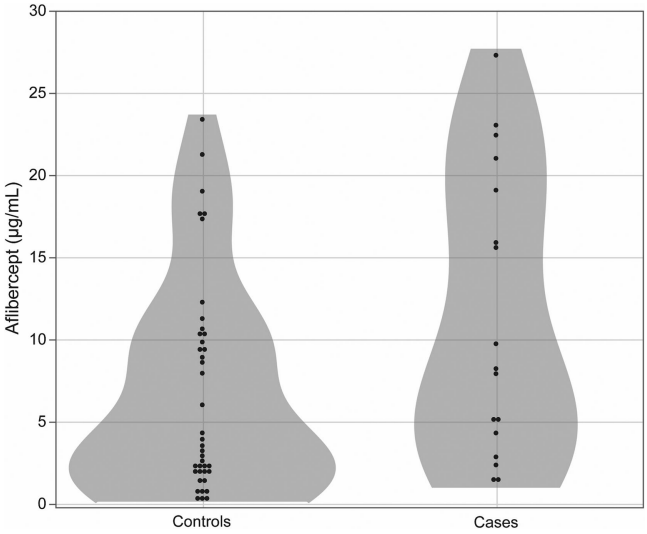

Figure 1. Distribution of aflibercept levels in the aqueous humor. Wilcoxon rank–sum test showed that there was no significant difference between controls and cases ($P = 0.053$).

Aflibercept Level

The distribution of aflibercept levels in controls and cases is illustrated in Figure 1. Although the

Table 1. Characteristics of Patients With nAMD

		Cases		
	Controls ($n = 40$)	Outflow Drugs ($n = 7$)	Inflow Drugs ($n = 10$)	*P*
Age (y), mean (IQR)[a]	73.6 (70.3–78.0)	71.3 (65.0–77.0)	76.1 (74.8–80.0)	0.24
Male sex, *n* (%)[b]	21 (53)	5 (71)	3 (30)	0.23
Axial length (mm), mean (IQR)[a]	23.83 (22.93–24.35)	23.85 (22.39–25.53)	23.73 (23.04–24.46)	0.99
IOP (mmHg), mean (IQR)[a]	14 (12–16)	13 (10–16)	15 (11–17)	0.78
Time from IVA to aqueous humor sampling (d), mean (IQR)[a]	29.9 (29.0–30.8)	29.1 (28.0–29.0)	29.3 (28.0–30.3)	0.40
Subtypes, *n*[b,c]	12, 2, 3, 23	4, 0, 0, 3	6, 1, 0, 3	0.43
Phakia, *n* (%)[b]	27 (68)	6 (83)	6 (60)	0.52

[a]Kruskal–Wallis test.
[b]Pearson's χ^2 test.
[c]Subtypes: macular neovascularization (MNV) type 1, MNV type 2, MNV type 3, and polypoidal choroidal vasculopathy.

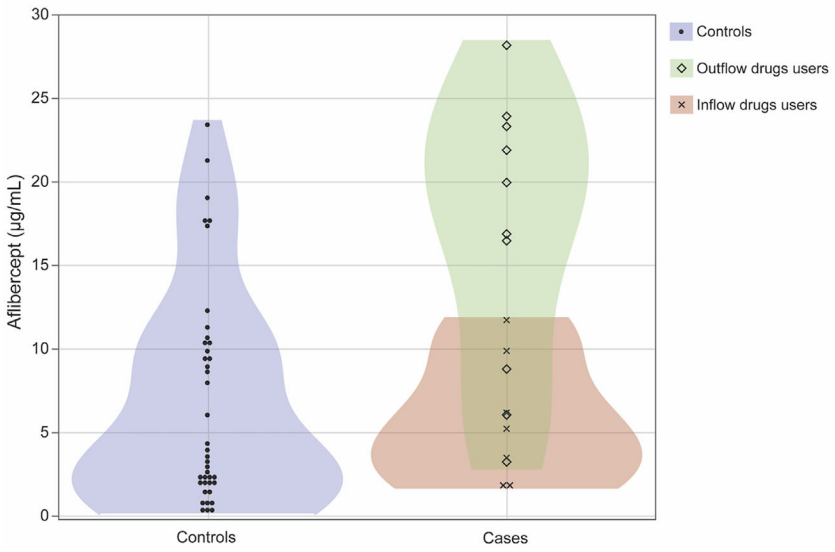

Figure 2. Distribution of aflibercept levels in the aqueous humor for users of each type of eye drop. Scatterplot of the aflibercept level in controls and cases who used eyed drops, including outflow drugs (*green markers*), and inflow drugs (*red markers*). A Kruskal–Wallis test showed a significant difference among the control, inflow, and outflow drugs ($P = 0.0075$). Dunn's test showed that aflibercept levels in the aqueous humor were significantly higher in cases using inflow drugs compared to both controls and cases using outflow drugs ($P = 0.0085$ and 0.044, respectively).

Table 2. Multivariate Associations Between Aflibercept Levels and Glaucoma Eyedrop Use

			Univariate		Multivariate Adjusted			
					Model 1[b]		Model 2[c]	
	n	Aflibercept (µg/mg), Mean (IQR)	Mean Difference (95% CI)	*P*[a]	Mean Difference (95% CI)	*P*	Mean Difference (95% CI)	*P*
Control	40	6.83 (1.94 to 10.34)	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Outflow drugs	7	4.71 (1.44 to 8.17)	6.78 (−2.89 to 16.44)	0.99	2.89 (−1.36 to 7.13)	0.19	1.59 (−2.53 to 5.71)	0.45
Inflow drugs	10	15.95 (7.20 to 22.57)	15.19 (5.08 to 25.28)	**0.0085**	8.36 (3.37 to 13.34)	**0.0020**	8.08 (3.22 to 12.94)	**0.0021**

Outflow drugs included PGAs, and inflow drugs included beta-blockers, alpha-2 agonists, and CAIs.
Values with $P < 0.05$ are bolded to indicate statistical significance.
[a]Dunn's test (compared with controls).
[b]Model 1 was adjusted for age, sex, and axial length.
[c]Model 2 was adjusted for age, sex, axial length, and time from intravitreal aflibercept injection to aqueous humor sampling.

aflibercept level in the aqueous humor was slightly higher in cases than in controls, there was no significant difference between controls and cases ($P = 0.053$). Figure 2 shows the distribution of aflibercept levels in controls and cases using each eyedrop type.

Table 2 shows the mean (IQR) aflibercept levels in the aqueous humor in controls, outflow drug users, and inflow drug users: 6.83 µg/mL (1.94–10.34), 9.93 µg/mL (2.58–17.44), and 15.95 µg/mL (7.20–22.57), respectively. A Kruskal–Wallis test showed a significant difference among the control, inflow, and outflow drugs ($P = 0.0075$). Dunn's test showed that aflibercept levels in the aqueous humor were significantly higher in cases using inflow drugs compared to both controls and cases using outflow drugs ($P = 0.0085$ and $P = 0.044$, respectively).

Multiple regression analysis model 1, adjusted for the parameters age, sex, and axial length, showed that inflow drug use (CAI, beta-blocker, and alpha-2 agonist) was significantly associated with a high aflibercept level ($P = 0.0020$). Multiple regression analysis model 2, adjusted for the parameters age, sex, axial length, and time from intravitreal aflibercept injection to aqueous humor sampling, also showed that inflow drug use was significantly associated with a high aflibercept level ($P = 0.0021$) (Table 2).

Predicted Biological Activity

Based on previous studies suggesting a half-life of aflibercept between 9 and 11 days,[12–15] our predictive model indicated that it would take approximately 141.7 to 167.4 days for the concentration to reach the same level as A_{84}. Thus, the duration of the biological activity of aflibercept was extended by approximately 57 to 83 days.

Discussion

This study showed that aflibercept levels in the aqueous humor 1 month after the first aflibercept injection were higher in inflow drug users (drops including a CAI, beta-blocker, and alpha-2 agonist) than in controls ($P = 0.0085$). Multivariate analysis models 1 and 2 revealed that inflow drug users were significantly associated with a high aflibercept level ($P = 0.0020$ and 0.0021, respectively). These eye drops extended the duration of the biological activity of aflibercept by about 57 to 83 days.

CAIs, beta-blockers, and alpha-2 agonists are widely used to lower intraocular pressure by reducing aqueous humor secretion, whereas PGAs and alpha-2 agonists are also used to reduce resistance to aqueous humor outflow. Cases who used inflow drugs had a higher aflibercept level in the aqueous humor. Although the time from aflibercept injection to aqueous humor sampling was significantly shorter in PGA and outflow drug users, cases who used an outflow drug did not have a higher aflibercept level in the aqueous humor. Multivariate analysis demonstrated a significant association between inflow drug use and higher aflibercept levels in the aqueous humor, regardless of the time from aflibercept injection to aqueous humor sampling. The primary mechanism of aqueous humor outflow is pressure dependent. The outflow drugs can alleviate impaired outflow in glaucoma but have minimal effects on aqueous humor turnover. In contrast, inflow drugs lower intraocular pressure and slow aqueous humor turnover, potentially delaying the elimination of anti-VEGF agents from the human eye. Although the anatomy of the rabbit outflow apparatus differs significantly from that of humans, a previous report using rabbit eyes demonstrated that brinzolamide and maleate fixed-combination eye drops significantly extended the ocular residence time of intravitreally injected aflibercept.[16] The present study demonstrates the potential clinical use of topical eye drops to extend the ocular half-life of anti-VEGF agents.

It is difficult to determine the exact vitreous pharmacokinetics of anti-VEGF drugs. In this study, the vitreous pharmacokinetics of anti-VEGF drugs were based on the notion that anti-VEGF drugs passively diffuse into the aqueous humor and are released into the systemic circulation. When an inhibitor of aqueous humor secretion is used, the slower dilution rate of the drug concentration reaching the aqueous humor results in a higher concentration in the aqueous humor. Consequently, the vitreous–aqueous concentration gradient is expected to decrease, which may also affect the half-life in the vitreous. Previous reports have shown that the one-compartment model explains the measured values.[17,18] This model supports the possibility that a slower aqueous secretion might lead to slower vitreous fluid turnover. However, there are still many factors that might affect the vitreous pharmacokinetics of drugs, such as how eye movements such as saccades affect the convective–diffusive behaviors of intravitreally delivered drugs[19–21] and the retinal–choroid–sclera pathway.[22,23] The mechanism discussed in the current study should be confirmed experimentally or by simulation using a model.[24–27]

This study has several limitations. Although the study period was 7 years, a relatively small number of cases from two institutions were analyzed; approximately only 3% of Japanese patients have already started glaucoma treatment when they start nAMD treatment,[28] and the sample size of this study was limited by the inclusion criteria. Second, all cases were glaucoma patients and might have had altered aqueous humor circulation. We attempted to perform a sensitivity analysis for cases after excluding those who used one or more eye drops, and there was one case who used each type of eye drop except PGA. Because seven cases used multiple eye drops, the impact of a single type of eye drop on the aqueous humor could not be accurately assessed. Given that eye drops used to lower intraocular pressure have different mechanisms of action, either by reducing aqueous humor production or decreasing outflow resistance, we opted not to analyze the data based on eyedrop classes. We conducted multivariate analysis to account for the effects of the use of multiple eye drops and it was challenging to fully assess the impact of each individual eye drop. Additionally, this study evaluated only the aflibercept level in the aqueous humor. Although the results showed that inhibition of aqueous humor secretion via eye drops extended the half-life of the intravitreally injected drugs, it is not clear whether this concentration difference affected the clinical outcome. Finally, all cases and controls were Japanese people, and the study might be influenced by regional differences among the Japanese population. Further study including a focus on clinical outcomes is needed.

This study showed that aflibercept levels in the aqueous humor 1 month after the first aflibercept injection were higher in case patients who used eye drops, including a CAI, beta-blocker, or alpha-2 agonist, than in control patients. Our findings suggest that decreasing aqueous humor secretion might extend the half-life of intravitreally injected drugs. The combination of these eye drops might reduce the number of injections needed for patients requiring intravitreal injections of drugs.

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