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ORIGINAL RESEARCH

Changes in Aqueous Humor Cytokine Profile Following Intravitreal Brolucizumab Injection

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Purpose: Intravitreal brolucizumab, approved for neovascular age-related macular degeneration (nAMD), may trigger immune responses leading to intraocular inflammation (IOI) by increasing pro-inflammatory cytokines. This study evaluates cytokine levels in the aqueous humor of patients before and after intravitreal brolucizumab injection.

Patients and Methods: Fourteen eyes of fourteen participants with nAMD or polypoidal choroidal vasculopathy (PCV) and who received intravitreal brolucizumab injection were included. Aqueous humor was collected before and 1 month following the injection. The aqueous cytokine profile was analyzed using a bead-based multiplex immunoassay. Paired *t*-test and Wilcoxon-signed rank test were used to analyze the results.

Results: Ten eyes were diagnosed with PCV, and four were nAMD. The aqueous IL-8 and IL-22 levels were significantly increased after intravitreal brolucizumab injection with a mean change of 18.2 ± 32.57 pg/mL (95% CI −0.61 to 37.01, p = 0.04) and 15.46 ± 24.14 pg/mL (95% CI 1.53–29.40, p = 0.03), respectively. VEGF-A was significantly decreased with a mean change of −915.4 ± 831.72 pg/mL (95% CI −1395.62 to −435.18, p < 0.01). One patient was diagnosed with IOI, and the cytokine profile showed increased in aqueous pro-inflammatory cytokines (Exotaxin, G-CSF, IL-8, IL-10, IL-22, IL-10, MCP-1 and TNF- α) and decreased in VEGF-A level compared with baseline.

Conclusion: Our study demonstrated a significant increase in aqueous pro-inflammatory cytokines in eyes treated with intravitreal brolucizumab. Nearly all eyes studied showed no clinical signs of intraocular inflammation. The results suggested that type IV cell-mediated hypersensitivity could play a role in IOI following intravitreal brolucizumab injection.

Keywords: anti-VEGF, intravitreal injection, inflammation, hypersensitivity, intravitreal injection

Introduction

Neovascular age-related macular degeneration (nAMD) is a degenerative disease affecting the macular area of the retina and typically occurs in patients over 60 years old.[1] In 2020, nAMD was one of the leading causes of blindness worldwide,[2] and it was estimated that the number of patients with AMD globally will reach 288 million by 2040.[3] The current standard treatment for nAMD is intravitreal injection of anti-vascular endothelial growth factor (anti-VEGF), and this treatment has been shown to improve patients' visual acuity and alleviate the severity of the disease.[4] Newer medications primarily aim to extend the duration of anti-VEGF inhibition, minimizing the need for frequent injections while also broadening the range of biological targets for inhibiting proangiogenic cytokines.[5]

Brolucizumab is an anti-VEGF agent approved by the US FDA for treating nAMD. It is a humanized single-chain variable fragment antibody targeting at VEGF-A.[6] Despite its efficacy, brolucizumab-associated ocular adverse events have been increasingly reported, including sterile intraocular inflammation (IOI), retinal vasculitis, and the most severe form, occlusive retinal vasculitis.[7–9] The symptoms of acute-onset IOI typically occur within 5 days after injection.

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Patients with IOI usually experienced decreased visual acuity, vitreous cells, anterior chamber cells, and floaters,[10] and treatment for IOI often involves observation or topical corticosteroids.[11] IOI may be accompanied by retinal vasculitis and occlusive retinal vasculitis, which leads to significantly worse visual acuity and prognosis.[8,9,12] Based on current evidence, the prevalence of these reactions in patients using brolucizumab is approximately 3.3–4.4%[7] compared to other anti-VEGFs at between 0.05% and 4.0%.[10] To date, the underlying pathophysiology of these reactions remains unclear.

Measuring cytokines level or cytokines profile in aqueous humor has been beneficial in explaining the etiology and pathophysiology of several diseases, including, diabetic retinopathy,[13] and nAMD.[14] Previous studies on aqueous cytokine profiles following brolucizumab injection have shown increased pro-inflammatory cytokine levels compared with other agents.[15,16] Terao et al compared aqueous cytokines in eyes with IOI after initial brolucizumab treatment to control eyes, including those without IOI after brolucizumab and eyes treated with aflibercept. They found that several cytokines, particularly interleukin-6 (IL-6), IL-8 and IL-10, were significantly elevated in the eyes with IOI following brolucizumab treatment compared to controls. IL-6 is linked immune cells activation and the production of other cytokines, while IL-8 acts as a chemotactic factor that promotes neutrophil migration, contributing to the inflammatory response in affected tissues. On the other hand, IL-10, normally considered as an anti-inflammatory cytokine, might be increased to modulate the inflammation. They also compared participants without IOI following brolucizumab to aflibercept, and they found that there was no significant difference in all cytokines except for lower aqueous VEGF level in participants' brolucizumab without IOI.[16] Hashimoto et al studied eyes receiving brolucizumab and reported that levels of the aqueous pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and IL-1, were significantly higher in eyes with IOI following brolucizumab injections compared to those without IOI before brolucizumab initiation.[15] However, none of the previously published studies compared the cytokine levels before and after brolucizumab injections.

We hypothesized that intravitreal brolucizumab injection could trigger an immune response inducing inflammation through increased pro-inflammatory cytokines in the eye, leading to IOI. Therefore, we aimed to assess cytokine levels in the aqueous humor of patients before and after intravitreal brolucizumab injection.

Methods

Study Participants

This study is a comparative observational study of cytokines level in aqueous humor samples before and after intravitreal brolucizumab injection. This study was approved by the institutional review board of Faculty of Medicine, Chulalongkorn University, in accordance with the Declaration of Helsinki, and was registered in the Thai Clinical Trial Registry (TCTR20220511001). Written informed consent for enrollment and public data publication were obtained from all patients before their enrollment to the study.

We included consecutive cases of naive and previously treated patients with nAMD and polypoidal choroidal vasculopathy (PCV) diagnosed by fundus fluorescein angiography (FFA), indocyanine green angiography (ICGA) and/or optical coherence tomography (OCT) (Spectralis, Heidelberg Engineering, Heidelberg, Germany) and received intravitreal brolucizumab injection. Patients with other retinal diseases, history of or active ocular inflammation or infection in either eye, history of or active systemic autoimmune diseases or cancer, hypotony (intraocular pressure < 5 mmHg), significant peripheral corneal thinning, iridocorneal touch, and corneal haze obscuring lens and iris details were all excluded from the study.

Aqueous Humor Collection and Intravitreal Injections

Approximately 0.2 mL of aqueous humor of participants was collected from each participant in sterile tubes by anterior chamber paracentesis with a 30-gauge needle and a 1.0 mL syringe prior to the intravitreal injection (IVI) of brolucizumab. Aseptic technique was strictly applied throughout the procedure. 10% povidone-iodine solution was applied on the periocular skin, upper and lower eyelids, and eyelid margin, and 5% povidone-iodine solution was applied to the conjunctiva as eye drop. IVIs were done by using a pre-filled 30-gauge needle with 6 mg (0.05 mL of 120 mg/mL solution) of brolucizumab.[17] Both anterior chamber paracentesis and IVI were performed by a retinal specialist (WK).

According to the treatment regimen, patients with nAMD and PCV and received intravitreal brolucizumab injection with a 4-week interval between each dose.[18] The first collection was performed before the injection of the initial dose of brolucizumab at week 0. The second collection was done on the day of the second dose of brolucizumab at week 4. All the aqueous humor samples were immediately labelled, stored in a 4°C refrigerator, and transferred within 24 hours to a −80°C storage at the laboratory unit of the Department of Biochemistry, Faculty of Medicine, Chulalongkorn University before further analysis. Any remaining sample after analysis was discarded.

Aqueous Cytokine Analysis

The aqueous humor was analyzed using a bead-based multiplex immunoassay panel (MILLIPLEX® Human cytokine/chemokine/growth factor panel A, Merck EMD Millipore, Billerica, MA). The following cytokines are detectable by using this panel: interferon-gamma (IFN-γ), IL-1RA, IL-6, IL-8, IL-10, IL-17A, IL-18, IL-22, IFN-γ-inducible protein-10 (IP-10), monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein-1 alpha (MIP-1α), TNF-α, Exotaxin, RANTES, platelet-derived growth factor-AA (PDGF-AA), VEGF-A, fibroblast growth factor-2 (FGF-2), granulocyte-colony stimulating factor (G-CSF), epidermal growth factor (EGF), and transforming growth factor-alpha (TGFα). The results of each cytokine level will be reported as pg/mL.

Statistical Analyses

Clinical data were collected, including best-corrected visual acuity (BCVA), central subfield thickness (CST), anterior chamber cells, anterior vitreous cells, and presence of subretinal fluid (SRF), intraretinal fluid (IRF), and retinal pigmented epithelial detachment (RPED). Demographic data and multiplex immunoassay results were also collected for statistical analysis. Statistical analysis was done using SPSS version 29 (SPSS software, Chicago, IL). Shapiro–Wilk test was used to determine the normality of the data. Paired *t*-test was used for normal distribution data, and Wilcoxon-signed rank test was used for non-normal distribution data. Data are presented as mean ± standard deviation (SD). p-value less than 0.05 was considered as a statistical significance.

Results

In this study, we evaluated a cohort of 14 eyes in 14 participants with a mean age of 73.10 ± 9.07 years. The gender distribution within the cohort consisted of 8 females (57%) and 6 males (43%). As we included participants who received intravitreal brolucizumab injection, the diagnoses among patients varied. Four patients (29%) were diagnosed with nAMD, while the majority, 10 patients (71%), were diagnosed with PCV. In terms of laterality, 9 patients (64%) had an involvement of the right eye. The BCVA for the cohort was measured in logMAR, with a mean value of 0.39 ± 0.37. The overall clinical outcomes for each participant are detailed in Table 1.

Biomarker Analysis

VEGF-A level exhibited a significant decrease from baseline with a mean change of −915.40 ± 831.72 pg/mL (95% CI −1395.62 to −435.18, p = 0.001). IL-8 and IL-22 level also showed a significant increase from baseline with a mean change of 18.20 ± 32.57 pg/mL (95% CI −0.61–37.01, p = 0.04) and 15.46 ± 24.14 pg/mL (95% CI 1.53–29.40, p = 0.03), respectively. Other cytokines did not show statistically significant changes from baseline after an initial dose of brolucizumab. The results of cytokine profiles are summarized in Table 2, and the comparison of cytokine levels with significant changes is displayed in Figure 1.

Case with IOI

We observed one participant with IOI, case No.3, at one month following brolucizumab injection. This participant previously received two aflibercept injections and one dose of brolucizumab. Initially, BCVA of this case was 0.3 on logMAR scale, and there were SRF and RPED with CST at 321 μm. No signs of IOI were observed before the initial injection of brolucizumab. The patient reported floaters and eye redness 5 days following the second dose of brolucizumab. We observed anterior chamber cells, vitreous cells, and vitreous haze, but we did not observe any signs of retinal vasculitis or retinal vascular occlusion. The diagnosis of brolucizumab associated-with anterior uveitis was made, and the

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Table 1 Patient Characteristics and Treatment Outcomes

No.	Age	Sex	Underlying Diseases	Diagnosis	Duration of Disease (Years)	Interval Since Last Anti-VEGF Injection to Baseline Collection (Months)	Previous Anti-VEGF	BCVA (logMAR)		CST (μm)		OCT Findings	
								Pre-Br	Post-Br	Pre-Br	Post-Br	Pre-Br	Post-Br
1	74	M	HT, gout, IFG	PCV	0	N/A	Naïve	1	0.4	200	158	Serous RPED, SRF	Decreased SRF, decreased RPED
2	80	F	HT, Bronchiectasis	PCV	6	1	Bevacizumab x3, Aflibercept x24, Brolucizumab x1 [a]	0	0	213	206	Fibrovascular RPED, serous RPED, SRF	Decreased SRF, decreased RPED, stable fibrovascular RPED
3 [b]	68	M	no	nAMD	N/A [c]	1	Aflibercept x2, Brolucizumab x1 [a]	0.3	0.2	321	227	RPED, SRF	Resolved large RPED, decreased SRF
4	82	M	HT, DM, POAG	PCV	12	2	Aflibercept x15	0	0	274	264	Fibrovascular RPED, SRF	Fibrovascular RPED, resolved SRF
5	75	F	HT, old CVA	nAMD	1	2	Bevacizumab x4, Aflibercept x8	0.4	0.6	321	257	Serous RPED, SRF	Decreased SRF, decreased RPED
6	77	F	HT	PCV	6	2	Bevacizumab x3, Aflibercept x25	0.1	0.7	462	262	Fibrovascular RPED	Decreased fibrovascular RPED
7	66	M	HT, DLP, CAD	PCV	6	2	Bevacizumab x2, Ranibizumab x3, Aflibercept x33	1	1	445	449	SRF, RPED	Decreased SRF, decreased RPED
8	84	F	No	PCV	12	4	Bevacizumab x8, Ranibizumab x1, Aflibercept x28	0.4	0.2	194	189	Serous RPED, fibrovascular RPED, SRF	Decreased SRF, stable RPED
9	72	M	No	nAMD	0	4	Bevacizumab x2	0.2	0.2	293	294	Fibrovascular RPED	Fibrovascular RPED
10	73	F	HT	PCV	11	4	Bevacizumab x12, Ranibizumab x2, Aflibercept x27, Brolucizumab x2 [d]	0.9	0.9	326	437	Multilobulated RPED, large SRF	Slightly decreased SRF, RPED

11	48	F	No	PCV	N/A [c]	5	Aflibercept x3, Brolucizumab x4 [d]	0.1	0	240	249	SRF, fibrovascular RPED	Resolved SRF, multiple serous RPED
12	69	M	HT, DM, DLP	PCV	2	6	Bevacizumab x2, Aflibercept x7, Brolucizumab x3 [d]	0.1	0.1	279	322	RPED, SRF	Decreased SRF, decreased RPED
13	82	F	Anemia	nAMD	5	59	Aflibercept x5	0.2	0.4	238	216	RPED, SRF	Minimally decreased SRF, RPED
14	74	F	No	PCV	N/A [c]	N/A [c]	Unknown [c]	0.8	0.8	317	273	Fibrovascular RPED, serous RPED, SRF	Decreased SRF, decreased RPED

Notes: [a] The patient received an initial dose of brolucizumab 4 weeks prior to the enrolment. [b] The patient was diagnosed with IOI following brolucizumab. [c] The participants were referred from other centers and could not recall the duration of the disease and previous medication given. [d] The last dose of brolucizumab was given at least 4 months prior to the initial dose in this study.

Abbreviations: BCVA, Best-corrected visual acuity; CST, Central subfield thickness; CVA, Cerebrovascular accident; DLP, Dyslipidemia; DM, Diabetes mellitus; HT, hypertension; IFG, Impaired fasting glucose; IOI, Intraocular inflammation; IRF, intraretinal fluid; N/A, not applicable; nAMD, neovascular age-related macular degeneration; OCT, optical coherence tomography; PCV, Polypoidal choroidal vasculopathy; POAG, Primary open-angle glaucoma; Pre-Br, pre-brolucizumab injection; Post-Br, post-brolucizumab injection at 1 month; RPED, Retinal pigmented epithelial detachment; SRF, subretinal fluid.

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Table 2 Comparison of Cytokine Levels Before and After Intravitreal Brolucizumab Injection

	Pre-Brolucizumab	Post-Brolucizumab	Mean ± SD	95% CI	p-value
Exotaxin	6.89 ± 6.35	10.43 ± 12.38	3.55 ± 11.50	−3.09–10.19	0.27
FGF2	0 ± 0	2.66 ± 5.37	2.66 ± 5.37	−0.44–5.76	0.09
G-CSF [a]	19.79 ± 32.56	147.16 ± 264.10	127.37 ± 265.07	−25.68–280.42	0.08
IFN-γ	4.03 ± 1.85	4.24 ± 2.63	0.21 ± 3.04	−1.55–1.97	0.80
IL-1RA [a]	2.09 ± 2.76	3.57 ± 4.39	1.47 ± 5.50	−1.70–4.65	0.55
IL-6	185.67 ± 388.53	383.16 ± 378.86	197.49 ± 462.21	−69.39–464.36	0.13
IL-8 [a]	14.61 ± 8.08	32.81 ± 34.37	18.20 ± 32.57	−0.61–37.01	0.04
IL-10 [a]	1.37 ± 1.88	4.67 ± 9.44	3.30 ± 8.89	−1.84–8.43	0.25
IL-17A [a]	0.08 ± 0.21	0.40 ± 0.54	0.32 ± 0.58	−0.02–0.66	0.06
IL-18 [a]	0.54 ± 1.40	1.37 ± 4.50	0.82 ± 4.88	−2.00–3.64	0.92
IL-22	33.52 ± 13.47	48.98 ± 21.33	15.46 ± 24.14	1.53–29.4	0.03
IP10 [a]	1002.73 ± 2465.27	1191.28 ± 2137.10	188.56 ± 2856.83	−1460.93–1838.04	0.11
MCP1	2649.25 ± 1651.43	6742.28 ± 10961.29	4093.03 ± 9936.92	−1644.38–9830.44	0.15
MIP1 α	56.37 ± 16.18	59.67 ± 12.67	3.30 ± 15.90	−5.88–12.48	0.45
PDGFAA	175.63 ± 72.93	167.51 ± 61.58	−8.12 ± 69.38	−48.18–31.93	0.67
RANTES	0 ± 0	3.55 ± 13.30	3.55 ± 13.30	−4.12–11.23	0.34
TNF-α [a]	1.78 ± 2.02	4.99 ± 9.74	3.21 ± 8.44	−1.66–8.08	0.07
VEGF-A	1437.56 ± 560.52	522.15 ± 728.22	−915.40 ± 831.72	−1395.62 – −435.18	0.001

Notes: EGF and TGF-alpha were not detected, pre- and post-treatment. Cytokines were measured in pg/mL. [a]Wilcoxon-signed rank test.

patient was treated with only topical corticosteroid. At one month after the second dose of brolucizumab, BCVA was 0.2 logMAR, and we still observed signs of IOI: anterior chamber cells, vitreous cells, and vitreous haze without any signs of retinal vasculitis. The anterior chamber cells were decreased from 3+ to 2+, at that time, we discussed options with the patient and decided to increase the topical 1% prednisolone acetate eye drop to every 2 hours for 2 weeks which resulted in decreased of anterior chamber cells from 2+ to 0.5+ and no vitreous cells, vitreous haze or signs of retinal vasculitis at all. Therefore, we decided with the patient to taper topical corticosteroid until cessation. There was no relapse of IOI, and the treatment was switched to another anti-VEGF. SRF and RPED at one month decreased dramatically from the baseline, and CST was decreased to 227 μm. The cytokine profile of this participant at baseline was at the higher end of the cohort in various cytokines, such as, Exotaxin, G-CSF, IL-8, IL-10, IL-22, IL-10, MCP-1, and TNF- α, and showed an increase of more than 300% in G-CSF, IL1-RA, IL-6, IL-8, IL-10, and TNF-α, while VEGF was decreased from 688.76 to 5.77 pg/mL.

Discussion

In this study, we included participants who were receiving intravitreal brolucizumab injections. Our primary objective was to gain insight into how the cytokine profile changes after an initial dose of brolucizumab. Although nAMD affects the posterior segment of the eye, previous studies have shown that cytokine profiles in the aqueous humor are associated with those in the vitreous samples across various diseases, including nAMD.[19–21] Therefore, we evaluated aqueous

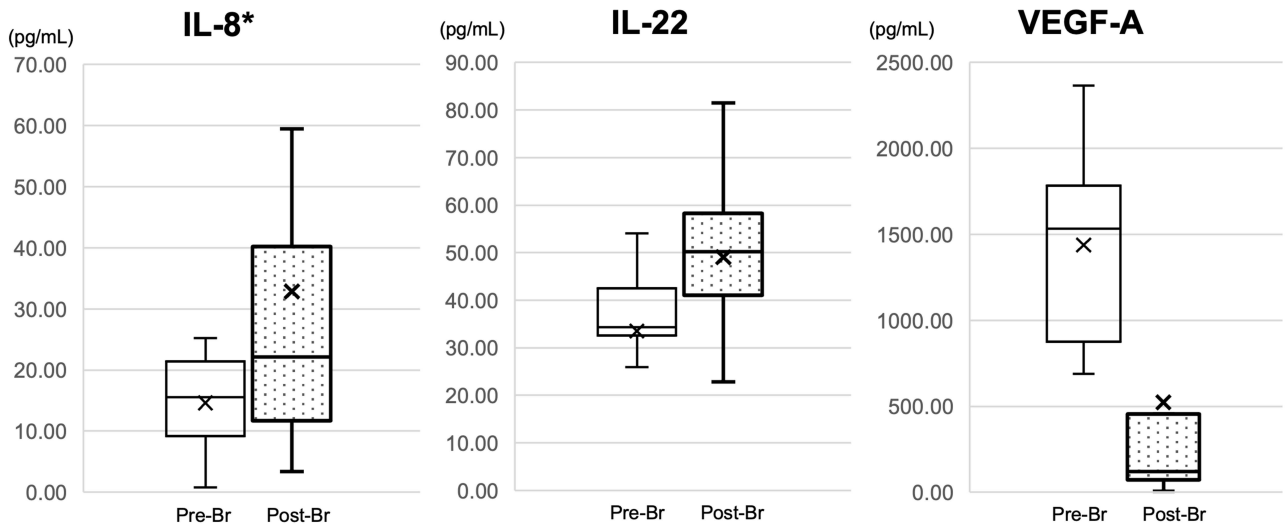

Figure 1 Comparison of cytokines with significant changes from baseline. X in each box represents the mean of each data set. Cytokines were measured in pg/mL. *Wilcoxon-signed rank test.
Abbreviations: IL, interleukin; Pre-Br, pre-intravitreal brolucizumab injection; Post-Br, post-intravitreal brolucizumab injection; VEGF, vascular endothelial growth factor.

humor samples in patients with nAMD which can represent vitreous samples. Despite the aqueous humor being collected four weeks after the previous injection, we observed some trends in the results.

VEGF-A plays a critical role in the pathogenesis of nAMD, particularly the development of choroidal neovascularization.[22] One of the significant findings was a decrease in VEGF-A levels after brolucizumab injection, which aligns with the expected mechanism of action of the drug. This result is consistent with those of other studies on different anti-VEGF agents.[13,23] Moreover, we observed 64% decreased from baseline in VEGF-A levels one month following initial brolucizumab injection. In previous studies in treatment-naïve eyes, VEGF-A levels decreased about 33–80% and 78% from baseline, one month after ranibizumab and aflibercept, respectively.[23,24] The substantial reduction in VEGF-A levels observed with brolucizumab may suggest its molecular efficacy in non-treatment-naïve cases.

As majority of our cases involve PCV and demonstrate a suboptimal response to prior anti-VEGF therapy, this can be attributed to several diseases-specific characteristics, including increased choroidal thickness, dilated choroidal vessels,[25] and choroidal vascular hyperpermeability.[26] Interestingly, beyond VEGF, other aqueous cytokines such as VCAM-1 and TNF-α have been identified as independent risk factors for suboptimal responses to anti-VEGF treatment.[27] Therefore, a comprehensive assessment of choroidal features and cytokine profiles may be crucial for predicting treatment outcomes and optimizing therapeutic strategies in patients with PCV.

The efficacy of brolucizumab was also evident. Following one dose of brolucizumab, we observed improvements in SRF and RPED in multiple cases, however the changes of CST were mixed. In the naïve case, we found that BCVA (1 to 0.4 logMAR), CST (200 to 158 μm), SRF and RPED were all improved from baseline after just one dose of intravitreal brolucizumab. For the case of IOI, both functional and anatomical outcomes improved dramatically from the baseline. We observed that decreased VEGF-A levels corresponded to clinical improvement in patients, suggesting a potential relationship between VEGF levels and clinical outcomes.

According to our observation in the study, the aqueous levels of IL-8 and IL-22 increased significantly from baseline, and both cytokines play a role in innate immune response and hypersensitivity reactions. IL-8, produced by T lymphocytes, involves recruiting neutrophils to inflammatory sites, chemotactic for basophils and T lymphocytes, and type IV cell-mediated hypersensitivity, which then activates neutrophil secretion in inflammatory cytokines. It is typically found only in inflammatory conditions.[15,28,29] IL-22, predominantly produced by T-helper cells (Th) 1, 17 and 22, can induce expression of inflammatory cytokines, for example, IL-1, IL-6, IL-8 and G-CSF, and chronic expression of IL-22 in normal tissue can result in production of cytokines, chemokines and other inflammatory signals, and ultimately, recruitment of pathologic effector cells to the inflamed tissues.[30] It is implicated in the pathogenesis of

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inflammatory bowel disease and psoriasis, both of which are associated with type IV cell-mediated hypersensitivity. Furthermore, IL-22 plays a significant role in the innate immune response. It affects the non-hematopoietic epithelial cells and fibroblasts in tissues, such as lung, liver and skin,[31] and also induces the production of neutrophil-attracting chemokines, thus enhancing defense mechanisms against bacterial and fungal infections.[32–34] Type IV cell-mediated hypersensitivity, also known as delayed-type hypersensitivity, mainly involves T cell antigen recognition, supported by other leukocytes, and resulted in effects on other cells.[35] Therefore, both IL-8 and IL-22 play a role in type IV hypersensitivity, but in different ways. Compared to previous studies, Terao et al found that the individual with IOI following brolucizumab injection had significantly higher IL-8 than those without IOI,[16] and Sun et al found that IL-8 levels also slightly increased from baseline after ranibizumab injection at one month; however, there was no statistical significance.[23] We hypothesize that there may be some degree of subclinical inflammation following brolucizumab injection, observable in the cytokine levels.

In addition to VEGF-A, IL-8 and IL-22, we did not observe significant changes in aqueous cytokine levels between baseline and post-injection levels. This lack of significant change could be attributed to the varying durations of the disease, prior treatments, the individual cytokine profiles of participants, or the individual response to brolucizumab, as most had been diagnosed and treated with multiple anti-VEGF agents for years before receiving brolucizumab. Despite these baseline differences, we expect that the before and after changes in cytokine levels should exhibit a similar trend.

Additionally, we encountered one case of intraocular inflammation following brolucizumab. This patient reported floaters 5 days after the second intravitreal brolucizumab injection and was treated as IOI with topical corticosteroids. We followed our protocol and collected aqueous humor for analysis 4 weeks after the injection, and at which time we still observed signs of IOI. The cytokine profile of this patient revealed an increase in pro-inflammatory cytokines, such as IL1-RA, IL-6, IL-8, IL-10, and TNF-α, accompanied by a dramatic decrease in VEGF levels, CST, SRF, and RPED. Despite weeks of treatment with topical corticosteroids, we continued to observe elevated pro-inflammatory cytokines and signs of IOI. The onset and presentation of IOI were consistent with a previous report,[36] and the increase in pro-inflammatory cytokines was also aligned with previous studies.[15,16]

Several hypotheses have been proposed regarding the pathophysiology of IOI following brolucizumab administration. First, the anti-drug antibody (ADA) might play a role in IOI, and this was also applied to other anti-VEGF agents.[10] The antigen–antibody complex, in this case the ADA and brolucizumab molecule complex, could aggregate in the eye, cause inflammation and tissue damage to the eye, and lead to type III immune complex hypersensitivity,[37] which could consequently cause vasculitis. Pre-existing ADAs were detected in treatment-naïve patients. Notably, ADA against brolucizumab was identified in 36–52% of naïve eyes compared with ADAs against ranibizumab or aflibercept at 0–3%.[12,38] This difference could be attributed to the single-chain structure of the brolucizumab molecule that could potentially mimic natural antigens.[39] Six percent of patients with ADA experienced IOI after intravitreal brolucizumab injections compared to 2% of patients without ADA,[40] thus suggesting a higher prevalence of IOI following brolucizumab. Second, IOI has been linked to hypersensitivity reactions, as lymphocytes, which are pivotal in type IV hypersensitivity, have been identified in the vitreous humor of patients with IOI.[41] These reactions usually take several days to manifest their characteristics features, which aligns with the reported onset of IOI.[11] Another hypothesis is that the smaller brolucizumab molecule might have higher affinity for VEGF and deeper retinal penetration, which potentially affecting blood flow in the retina and inducing more inflammation in the eye.[12]

The mentioned hypotheses could explain the case of IOI in this study. Given the previous treatment with brolucizumab, it is plausible that ADA may develop after the first injection, potentially inducing inflammation in the eye. The elevation of cytokines in this case, such as G-CSF, IL-6, IL-8, IL-22 and TNF-α, which are typically involved in type IV cell-mediated hypersensitivity reactions,[16] supports the type IV cell-mediated hypersensitivity hypothesis. Considering the onset of IOI in this patient, it corresponds to the immune process involving cell-mediated immune response, which generally requires days to activate lymphocytes and manifest the reaction. Consequently, this observation might emphasize the possibility that IOI could potentially be induced by the presence of ADA, subsequently signaling through the hypersensitivity pathway.

In a recent meta-analysis investigating the incidence of IOI following intravitreal anti-VEGF injections in nAMD, no significant difference was found in the risk of serious IOI across different anti-VEGF agents. However, compared to

aflibercept, brolucizumab was associated with a significantly higher incidence of generalized intraocular inflammation (hazard ratio = 6.24) and vitreous opacities (hazard ratio = 1.64).[42] Additionally, a study by Ma et al, utilizing data from the FDA Adverse Event Reporting System (FAERS), found that brolucizumab was linked to a higher incidence of retinal vasculitis and retinal artery occlusion compared to both aflibercept and ranibizumab.[43] These findings underscore the importance of carefully selecting anti-VEGF agents, especially in patients with a history of ocular inflammation, to minimize the risk of adverse inflammatory events and optimize treatment outcomes.

Our study has several limitations. First, the sample size was relatively small. While this small cohort provides valuable data, it limits both the statistical power and generalizability of the findings. Second, the participants in this study were predominantly non-treatment-naïve, meaning previous injections with other anti-VEGF agents may have altered their cytokine profiles. This lack of standardization regarding treatment history introduces significant heterogeneity into the findings. Third, we did not collect aqueous humor samples following all injections, as it might not have been necessary to perform intravitreal injections after the loading doses. However, according to the HAWK and HARRIER study, the effect of brolucizumab tends to plateau after the first dose,[18] leading us to assume that cytokine levels would follow a similar trend. Therefore, larger, prospective studies with stricter inclusion criteria, specifically focusing on treatment-naïve patients, are needed to address these issues.

Conclusion

In summary, our study demonstrated a significant increase in specific pro-inflammatory cytokines in the aqueous humor of eyes treated with intravitreal brolucizumab, although nearly all the studied eyes exhibited no clinical signs of intraocular inflammation. This observation suggests that the mechanism underlying brolucizumab-induced IOI may be related to type IV cell-mediated hypersensitivity. Future studies on other anti-VEGF agents should provide more insight about the IOI following intravitreal anti-VEGF injections.

Data Sharing Statement

The de-identified participant data that support the findings of this study are available from the corresponding author, WK, upon reasonable request after publication.

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Disclosure

The authors declare no conflicts of interest related to this study.

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