Intravitreal Anti-VEGF Injections Reduce Aqueous Outflow Facility in Patients With Neovascular Age-Related Macular Degeneration

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PURPOSE. We assess the effect of intravitreal anti-VEGF injections on tonographic outflow facility.

METHODS. Patients with age-related macular degeneration who had received unilateral intravitreal anti-VEGF injections were recruited into two groups, those with ≤10 and those with ≥20 total anti-VEGF injections. Intraocular pressure and tonographic outflow facility of injected and uninjected fellow eyes were measured and compared between groups. Risk factors for development of reduced outflow facility also were assessed.

RESULTS. Outflow facility was 12% lower in the injected eyes of patients who received ≥20 anti-VEGF injections, compared to contralateral uninjected eyes (P = 0.02). In contrast, there was no facility reduction for patients with ≤10 anti-VEGF injections (P = 0.4). In patients with ocular hypertension in the un.injected eye (IOP > 21 mm Hg, n = 5), the outflow facility of injected eyes was on average 46% lower (P = 0.01) than in the uninjected fellow eyes. This was significantly greater than the difference observed in patients with IOP ≤ 21 mm Hg in the uninjected eye (P = 2 × 10−4). In patients with ocular hypertension in the injected eye (n = 6) the differences in facility and IOP between contralateral eyes were significantly greater than in patients with IOP ≤ 21 mm Hg in the injected eye (P = 2 × 10−4 and P = 7 × 10−4, respectively).

CONCLUSIONS. Chronic anti-VEGF injections significantly reduce outflow facility in patients with AMD. The greatest facility reduction is observed in patients with baseline ocular hypertension. Ophthalmologists who administer anti-VEGF injections should be aware of these findings and monitor patients closely for changes in IOP or evidence of glaucoma, especially in those with pre-existing ocular hypertension.

Keywords: anti-VEGF, aqueous outflow facility, ocular hypertension, intraocular pressure
Contribute to decreased tonographic outflow facility including pre-existing ocular hypertension.

**METHODS**

**Patient Enrollment**

This study followed the tenets of the Declaration of Helsinki and was approved by the Duke University Institutional Review Board. All subjects gave written informed consent before study inclusion. Patients aged >50 years with a history of monocular NVAMD treated with intravitreal anti-VEGF injections were recruited from the Duke Eye Center Retina Clinics. Since mean values of outflow facility and IOP are not significantly different between paired eyes of normal (nonglaucomatous) individuals, we recruited patients receiving unilateral anti-VEGF injections to compare injected versus fellow uninjected eyes. In this manner each patient served as his or her own control for analyses. Patients were recruited into two groups: a low injection (<10 injections) and high injection (≥20 injections) group. Exclusion criteria were the same for injected and uninjected fellow eyes and are listed in Table 1.

Demographic information, including patient age, sex, ethnicity, ocular and medical history, ocular medications, and number and type of intravitreal anti-VEGF injections, was collected.

**Outflow Facility Measurements**

Intraocular pressure and tonographic outflow facility measurements were performed before a scheduled injection and at least 4 weeks after the last intravitreal injection. An average of 3 IOP measurements per eye were obtained using an iCare Rebound (Tiotat Oy, Helsinki, Finland) tonometer without topical anesthesia. The patient then was placed in the supine position and topical proparacaine was administered to both eyes. The outflow facility of each eye was measured by a single investigator (ICW) using an electronic Schiotz tonograph (V-Mueller and Co., Chicago, IL, USA). A 4-minute tracing was recorded using a 5.5 g weight. The outflow facility was calculated using standard interpretation methods where a best fit line along the tonographic tracing was assigned to determine the starting and ending points. All tracings were measured in a masked fashion by one investigator (RA). Tonography tracings with significant irregularities from patient blinking or eye movement, or tracings without ocular pulsations for >30 seconds were considered technically unsatisfactory and excluded from analysis.

**Statistical Analysis**

Demographic data for low and high injection groups were compared using a χ² square analysis. Differences between IOP and tonographic outflow facility in contralateral eyes were compared using paired t-tests. Unpaired t-tests were used to analyze the differences in outflow facility and IOP between groups. A P value <0.05 was considered statistically significant.

The sample size required to detect a 15% difference in tonographic outflow facility between injected and uninjected eyes in each group was calculated. Assuming a mean outflow facility of 0.23 ± 0.05 µL/min/mm Hg reported for our older patient demographic, it was estimated that 20 patients would provide an 80% chance to detect a 15% difference in the outflow facilities for each group (paired t-test, α = 0.05, β = 0.20).

**RESULTS**

Of 46 patients recruited for this study, 22 were in the low injection group and 24 in the high injection group. All patients had unilateral injections of bevacizumab (n = 2), ranibizumab (n = 1), aflibercept (n = 11), or a combination (n = 32). Four patients were excluded from the analysis (2 in the ≤10 and 2 in the ≥20 injection groups) due to poor quality tonographic tracings. Therefore, 20 and 22 patients were included in the final analysis for the low and high injection groups, respectively.

The baseline patient characteristics of the 2 groups are shown in Table 2. The patient demographics did not differ significantly between the 2 groups. As expected, the mean number of injections between groups was significantly different (6.3 ± 3.2 injections (range, 1–10) for the low injection group versus 30.3 ± 13.3 injections (range, 20–68) for the high injection group; P < 0.001). Average values of facility and IOP for each of the groupings are provided in Table 3.

The pairwise differences in outflow facility between injected and uninjected fellow eyes (ΔC) in the high injection group were compared using a 2 × 2 square analysis. Differences between IOP and tonographic outflow facility in contralateral eyes were compared using paired t-tests. Unpaired t-tests were used to analyze the differences in outflow facility and IOP between groups. A P value <0.05 was considered statistically significant.

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**Figure 1.** Difference in outflow facility (ΔC) between injected eyes and fellow uninjected eyes, grouped by ≤10 injections (n = 20, hollow circles) and ≥20 injections (n = 22, filled circles). Blue data points indicate patients who were normotensive in the uninjected eye (IOP ≤ 21 mm Hg); red data points indicate patients who were ocular hypertensive in the uninjected eye (IOP ≥ 21 mm Hg) in the uninjected eye. Green boxes indicate the inner interquartile range, while white lines through the boxes represent the mean values. ΔC < 0 indicates a lower outflow facility in the injected eye.

The IOP of the uninjected eye was used to reflect the hypertension status of the injected eye before anti-VEGF treatment. The mean outflow facility in injected eyes was on average 46% lower than that in uninjected fellow eyes for the group of ocular hypertensive cases (0.08 ± 0.02 vs. 0.15 ± 0.04 µL/min/mm Hg, P = 0.01, Fig. 1). In contrast, the average facility for the normotensive group was not different for injected versus uninjected fellow eyes (0.14 ± 0.04 vs. 0.15 ± 0.05 µL/min/mm Hg, respectively; ΔC = 0.00 ± 0.03 µL/min/mm Hg; P = 0.9). The difference in the average ΔC between the ocular hypertensive and normotensive groups was highly significant (P = 2 × 10^-4). The difference in facility between injected and uninjected eyes for the ocular hypertensive group, however, did not coincide with a difference in IOP (ΔP = -0.7 ± 3.5 mm Hg, P = 0.66). Similarly, no difference in IOP between injected and uninjected eyes was observed for the normotensive group (ΔP = -0.1 ± 2.4 mm Hg, P = 0.88). This suggests that a reduction in outflow facility in an otherwise healthy eye is not necessarily sufficient to induce ocular hypertension, possibly due to homeostatic mechanisms or secondary effects of anti-VEGF that act on other aspects of aqueous humor dynamics.

Patients who are ocular hypertensive in the injected eye (IOP > 21 mm Hg) are at risk of glaucomatous damage due to anti-VEGF therapy. In all of these patients (n = 6), IOP was greater in the injected eye compared to the contralateral uninjected eye (ΔP = 3.2 ± 2.4 mm Hg, P = 0.021), indicating that regardless of whether these patients were originally ocular hypertensive, anti-VEGF treatment led to a further elevation in IOP. The mean outflow facility in these injected eyes also was significantly lower than in the uninjected fellow eyes (0.095 ± 0.05 vs. 0.15 ± 0.05 µL/min/mm Hg, respectively; ΔC = -0.06 ± 0.04 µL/min/mm Hg, P = 0.02).

As shown in Figure 2, all cases of ocular hypertension in the injected eye (indicated by circled data points) fell in the lower right quadrant, indicating that these individuals exhibited elevated IOP and decreased facility in the injected eye, relative to the fellow uninjected eye. In contrast, cases without ocular hypertension in the injected eye were normotensive in the fellow eye (IOP > 21 mm Hg, n = 37). The IOP of the uninjected eye was used to reflect the status of the injected eye before anti-VEGF treatment. The mean outflow facility in injected eyes was on average 46% lower than that in uninjected fellow eyes for the group of ocular hypertensive cases (0.08 ± 0.02 vs. 0.15 ± 0.04 µL/min/mm Hg, respectively; ΔC = -0.07 ± 0.03 µL/min/mm Hg; P = 0.01, Fig. 2). In contrast, the average facility for the normotensive group was not different for injected versus uninjected fellow eyes (0.14 ± 0.04 vs. 0.15 ± 0.05 µL/min/mm Hg, respectively; ΔC = 0.00 ± 0.03 µL/min/mm Hg; P = 0.9). The difference in the average ΔC between the ocular hypertensive and normotensive groups was highly significant (P = 2 × 10^-4). The difference in facility between injected and uninjected eyes for the ocular hypertensive group, however,
These findings suggested that baseline ocular hypertension, therefore, may be a greater risk factor for development of impaired outflow facility than number of anti-VEGF injections. This result also may explain why only a subset of patients receiving intravitreal anti-VEGF injections exhibit elevated IOP following chronic exposure. The most dramatic decreases in outflow facility among paired eyes were observed in subjects who had ocular hypertension, defined by IOP > 21 mm Hg, in either the injected or uninjected eye. This suggested that the patients who are most at risk appear to be those who start anti-VEGF treatment with an unhealthy trabecular meshwork, which typically is associated with ocular hypertension. Because the trabecular meshwork already may be stressed in these patients, the additional burden of anti-VEGF therapy increases the risk for further IOP elevation. This burden is imposed, presumably, by anti-VEGF compounds disrupting endogenous VEGF signaling involved in regulating outflow through the trabecular meshwork, as described in our companion study.22 Therefore, it may be prudent to establish the baseline IOP before starting intravitreal anti-VEGF injections, to identify those patients who may be at higher risk for these adverse consequences.

Our findings were consistent with the retrospective studies examining the incidence of sustained IOP elevation following anti-VEGF injections. Most studies reported an incidence of approximately 3% to 11% for development of sustained IOP elevation after anti-VEGF injection.16 In an analysis by Hoang et al.,11 the risk for sustained elevation of IOP was greatest in patients with ≥29 injections compared to patients with ≤12 injections (odds ratio [OR], 16.1; P = 0.0088). Similarly, we observed a significant decline in aqueous outflow facility in patients with ≥20 injections where the mean number was approximately 30 injections. In the two studies that did not report an increased incidence of sustained IOP elevation following anti-VEGF injection, the mean number of injections was 8.4 and 9.5.20,25 This also is consistent with our findings that outflow facility is more significantly decreased in patients with ≤10 total injections. These studies paralleled our own observations regarding reduced outflow facility by suggesting that, in the case of sustained IOP, increased number of injections is associated with greater risk of an adverse event. The exact mechanism of outflow reduction remains unknown. Our companion study (VEGF as a Paracrine Regulator of Conventional Outflow Facility) demonstrates that endogenous VEGF expression in the trabecular meshwork is a paracrine regulator of conventional outflow facility, which may be perturbed by the anti-VEGF agents themselves.22 Other studies have suggested that a number of potential mechanisms may contribute to IOP elevation, which include inflammation, obstruction by particulates in the injectate, or by secondary angle closure.17,19 Because the patients in this study were being treated for AMD and not glaucoma, gonioscopy was not performed to evaluate the status of angle structures. This
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would be helpful in future studies to better characterize mechanisms contributing to development of ocular hypertension in this patient population. Lastly, whether this decrease in outflow facility changes following cessation of anti-VEGF treatment is unknown. The vast majority of patients in this study received anti-VEGF injections on a regular schedule, typically every 4 to 6 weeks. There were too few patients with longer injection intervals to analyze the effect of injection spacing on outflow facility. A limitation of our study is the lack of preinjection, baseline data on a number of our subjects. Many age-related macular degeneration (AMD) patients had received bilateral anti-VEGF therapy, so they were excluded from the study. In those who met the unilateral criterion, information on baseline IOP was limited or not available because treatment was initiated at other institutions. For this reason, we used the IOP of the uninjected eye as an estimate of baseline, preinjection status of both eyes. Therefore, an IOP of $\geq 21$ mm Hg in the uninjected eye was assumed to represent a baseline, preinjection status of ocular hypertension. A number of studies report monocular treatment trials of glaucoma medications to assess efficacy, where the untreated eye is used as a control for comparison.

However, anti-VEGF is known to exit the eye via the conventional outflow tracts and can be measured systematically following intravitreal injection. Therefore, unilateral injection might have effects on the contralateral eye. Several studies in diabetic macular edema have reported that injection into one eye is associated with improved macular edema in the fellow uninjected eye. Yet, even if the anti-VEGF medication affected the uninjected eye, the magnitude of the effect on outflow facility would be expected to be the same or less. Therefore, if the fellow uninjected eye were exposed to anti-VEGF, it would likely reduce rather than increase the asymmetry between paired eyes, making this analysis more conservative. Regardless, prospective, longitudinal studies with complete preinjection and follow-up data will be needed to corroborate the findings from this study.

The mean outflow facilities in our study were lower than expected compared to other published reports for older patients that had a mean age of approximately 60 years. However, tonographic outflow facility is known to decrease with age. The mean age of patients in our study was approximately 15 years greater than the mean age of patients in the prior studies that were referenced.

A number of studies have reported on the IOP lowering effect of cataract surgery and some suggest that this is due to an increase in outflow facility. This could potentially confound the facility comparisons in subjects with pseudophakia, especially if only one eye is pseudophakic. However, only 4 subjects were unilaterally pseudophakic. The remaining subjects were either bilaterally phakic or bilaterally pseudophakic.

In all four unilaterally pseudophakic patients, the pseudophakic eye also was the injected eye. Assuming outflow facility was increased following cataract surgery, the effect of pseudophakia in the injected eye would tend to decrease the difference in outflow facility between injected and uninjected eyes, and, therefore, oppose the observed reduction of outflow facility in eyes receiving injections. To further address this important issue, we performed an analysis limited to subjects who were bilaterally phakic. In the low injection group, there still was no significant difference in outflow facility between eyes ($\Delta C = 0.00 \pm 0.04 \mu L/min/mm Hg; P = 0.73, n = 11$), and in the high injection group, outflow facility in the injected eyes was significantly lower, on average than in the contralateral, uninjected eyes ($\Delta C = -0.05 \pm 0.04 \mu L/min/mm Hg; P = 0.02, n = 12$). Therefore, it appears unlikely that lens status accounts for effects of anti-VEGF injections on outflow facility.

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