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Short-term effect on the ocular circulation induced by unilateral intravitreal injection of aflibercept in age-related maculopathy

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ABSTRACT.

Purpose: **Intravitreal injection of anti-vascular endothelial growth factor (anti-VEGF) is the standard treatment for neovascular age-related macular degeneration (AMD). As VEGF is a physiological key player for regulating retinal vascular tone, questions have been raised whether the application of anti-VEGF could induce alterations in ocular perfusion.**

Methods: **The study included 20 eyes from 20 Caucasian patients with unilateral neovascular AMD and 20 fellow eyes. All eyes were treated with standard intravitreal injection of aflibercept (IVA). Measurements of blood flow at the optic nerve head (ONH) and the choroid were performed with laser speckle flowgraphy (LSFG). The intraocular pressure (IOP), systolic and diastolic blood pressure, heart rate, mean arterial pressure (MAP) and ocular perfusion pressure (OPP) were analysed. Measurements were performed at baseline and repeated immediately after the injection and 30 and 45 min later.**

Results: **Mean time between injection of aflibercept and first follow-up was 8:56 ± 4:25 min. The injection led to significant rise in IOP. In the injected eyes, mean blur rate (MBR, i.e. a relative measure of perfusion and the main outcome parameter of LSFG) within the major vessels of the ONH as well as at the entire ONH region decreased significantly ($p < 0.001$). No change in MBR was observed in the fellow eye. Choroidal blood flow was maintained stable in both eyes.**

Conclusion: **Intravitreal injection of aflibercept (IVA) led to a short-term reduction in perfusion only in the treated eye. This was independent from IOP, indicating a direct pharmacological effect. No changes in choroidal perfusion were observed during the first 45 min after the injection.**

Key words: aflibercept – age-related macular degeneration – anti-VEGF – laser speckle flowgraphy – ocular perfusion

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Introduction

Age-related macular degeneration (AMD) is one of the main causes of irreversible visual loss worldwide (Bourne et al. 2018). Neovascular (exudative or wet) AMD is characterized by angiogenesis originating from the choroidal or, less frequently, retinal circulation (Gupta et al. 2017). Vascular endothelial growth factor (VEGF) is known as a key player of choroidal neovascularization and plays a significant role in the pathologic upregulation of chorioretinal vascular permeability (McTigue et al. 1999).

With the introduction of anti-VEGF agents, targeted treatment strategies for AMD were realized in the last two decades and have become the first-line therapy for neovascular AMD (Zhang et al. 2017). Currently, three anti-VEGF drugs are available for intravitreal injection: ranibizumab (Lucentis; Genentech, Inc., San Francisco, CA, USA); bevacizumab (Avastin; Genentech, Inc.); and aflibercept (Eylea; Bayer HealthCare, Inc., Leverkusen, Germany, Europe and Regeneron Pharmaceutical, Inc., New York, US). Both ranibizumab and bevacizumab inhibit solely VEGF-A (Ferrara et al. 2006; Selid et al. 2014). In contrast, aflibercept is a recombinant fusion protein that inhibits VEGF-A,

VEGF-B and placental growth factor (Stewart 2012). All three anti-VEGF agents are to be administered by injection into the vitreous cavity. Thus, their effect is not only limited to the choroidal neovascularization (CNV) lesion in the macular region but the entire retina and optic nerve head (ONH) area are exposed to the drug. On a physiological level, VEGF acts as a vasodilator as it activates endothelial nitric oxide (NO) synthase, which produces the potent vasodilator NO (McTigue et al. 1999). Hence, it can be hypothesized that therapeutic VEGF inhibition might induce vasoconstriction in retinal and ONH vessels with a subsequent decrease in ocular perfusion. A range of studies have indicated that both bevacizumab (Ferrara et al. 2006; Sharei et al. 2010; Sugiyama et al. 2010; Stewart 2012; Selid et al. 2014; Zhang et al. 2017) and ranibizumab (Papadopoulou et al. 2009; Enaida et al. 2010; Sacu et al. 2011; Mendrinos et al. 2013; Kunikata et al. 2014; Okamoto et al. 2015; Sugimoto et al. 2017) induce alterations in ocular perfusion with sustained vasoconstrictive effects reported for up to 1 year after administration of the drug. The understanding of potential mid- and long-term vasoconstrictive side-effects with consequent chronic ocular hypoperfusion and potentially harmful structural or functional changes to the neurosensory retina and/or the ONH is yet to be established. Moreover, potential short-term alterations induced by intravitreal anti-VEGF agents must not be disregarded. Injection of ranibizumab into the vitreous has been shown to transiently increase the intraocular pressure (IOP), most pronounced in the first 3 min after injection with rapid decline (Sharei et al. 2010).

Laboratory research has indicated that aflibercept exhibits an almost 100 times greater binding affinity for VEGF compared to bevacizumab and ranibizumab as well as substantially prolonged binding activity (Aizawa et al. 2011). Hence, enhanced and sustained effects on ocular perfusion might be anticipated. Lately, reduced retrobulbar blood flow in the ophthalmic artery, central retinal artery and posterior ciliary artery was described 1 week after Intravitreal injection of aflibercept (IVA) (Gok & Kapti 2018). Significant vasoconstriction of the retinal arterioles was observed by another group following three monthly injections of aflibercept (Tetikoğlu et al. 2018).

Laser speckle flowgraphy (LSFG) is a promising technique for the measurement of ocular perfusion. This method enables two-dimensional, noninvasive measurements of perfusion at the ONH, the retina and the choroid (CHOR) using the laser speckle phenomenon (Sugiyama et al. 2010). Laser speckle flowgraphy (LSFG) has been used to assess the influence of intravitreal injection of bevacizumab and ranibizumab on ocular perfusion in patients with diabetic retinopathy, retinal vein occlusion or central serous chorioretinopathy (Enaida et al. 2010; Kunikata et al. 2014; Okamoto et al. 2015; Nagasato et al. 2016; Sugimoto et al. 2017).

The purpose of this study was to characterize for the first time the short-term changes in ocular perfusion induced by IVA in eyes with neovascular AMD by means of LSFG.

Materials and Methods

Patients

This prospective interventional study included 20 eyes of 20 Caucasian adult patients with unilateral neovascular AMD and 20 fellow eyes (FE). Patients were recruited consecutively from the medical retina clinic of the Kepler University Hospital Linz, Austria. The study protocol was reviewed and approved by the local ethics committee (Ethikkommission des Landes Oberösterreichs; registration number B-119-16) and followed the guidelines set forth in the Declaration of Helsinki. Written informed consent was obtained before inclusion in the study. All subjects underwent a comprehensive screening examination, including a slit-lamp examination with indirect funduscopy and measurement of IOP using the Goldmann applanation tonometry. Best-corrected visual acuity (BCVA) was assessed using the Jackson cross-cylinder method and the standard Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart.

The inclusion criteria were (1) age >50 years and (2) patients scheduled for three consecutive intravitreal injections (4-week intervals) of aflibercept for treatment of exudative AMD in one eye. The exclusion criteria included (1) active exudative AMD requiring treatment of both eyes, (2) ocular surgery (including intravitreal injection) during the 3 months preceding the study, (3) vitrectomized eyes, (4) ametropia >6 Dpt and (5) any relevant ophthalmic diseases/conditions that could interfere with LSFG measurements (e.g. glaucoma, ONH drusen, tilted disc). Subjects were instructed to abstain from alcohol and stimulating beverages containing xanthine derivatives (e.g. tea, coffee) 12 hr before the LSFG measurements, as these are known to potentially influence the results (Okuno et al. 2000). Measurements were performed in a quiet, dark room with the subject in sitting position.

Baseline measurements

Al subjects were evaluated carefully prior to the injection. Performed examinations included ETDRS BCVA and the Goldmann applanation tonometry. Optical coherence tomography (Spectralis; Heidelberg Engineering, Heidelberg, Germany) was employed to measure central retinal thickness (CRT). The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured at the upper arm with a manometer in the sitting position after a resting period of 5 min. The mean arterial pressure (MAP) was calculated as $MAP = DBP + 1/3\ (SBP - DBP)$, and the ocular perfusion pressure (OPP) as $OPP = 2/3\ MAP - IOP$.

Intervention

After instillation of topical anaesthetic (0.4% oxybuprocaine hydrochloride and lidocaine; own production), sterilization of the eyelid (Betaisodona Lösung®, 11% povidone–iodine, Mundipharma, Limburg, Germany), and instillation of 1.25% povidone–iodine drops, 2.0 mg/0.05 ml of aflibercept (Eylea) was injected into the vitreous cavity through a standard pars plana approach (3.5 mm posterior to the limbus) under sterile conditions. Surgeons were asked to evaluate the amount of reflux (0 = no reflux, 1 = minimal reflux, 2 = moderate reflux, 3 = excessive reflux).

Laser speckle flowgraphy

Laser speckle flowgraphy measurements were performed with the LSFG RetFlow

instrument (Nidek Co., LTD, Gamagori, Aichi, Japan) after pharmacological dilation of the pupil with 0.5% tropicamide eye drops (Mydriaticum Agepha Augentropfen; Agepha Ges.m.b.H., Vienna, Austria). The LSFG device consists of a fundus camera equipped with a diode laser at a wavelength of 830 nm and a digital charge-coupled device camera (750 × 360 pixels). A total of 118 images are acquired at a rate of 30 frames per second over a 4-second measurement period. The main output parameter of LSFG, mean blur rate (MBR), is derived from the pattern of speckle contrast produced by the interference of a laser scattered by blood cells moving in the ocular fundus. MBR was calculated for the total ONH area, defined by a ellipsoid region of interest (ROI; referred to as ONH-MA, 'mean MBR of all area'). By using the on-board software, the MBR was calculated in the large vessels within the ONH (ONH-MV, 'mean MBR of vascular area') and the tissue area containing the microvasculature (ONH-MT, 'mean MBR of tissue area'). For analysis of the CHOR, a square ROI was set (150 × 150 pixels) in a temporal location one optic disc diameter away from the ONH, without including the main retinal vessels as it was described recently (Aizawa et al. 2011). All ROI positions were saved and used for the follow-up measurements. Measurements of MBR have showed excellent repeatability in Caucasian subjects (Luft et al. 2016).

Table 1. Baseline characteristics of treated eyes (TEs) and fellow eyes (FEs) including biometric results (Student's *t*-test).

Characteristics	Number, mean ± SD	p
Sex		
Male	11	
Female	9	
Age (years)	75.1 ± 8.2	
SBP (mmHg)	143.1 ± 18.8	
DBP (mmHg)	82.1 ± 9.3	
HR (bpm)	71.7 ± 9.8	
MAP (mmHg)	102.4 ± 10.6	
MRSE (dpt)		
TE	−0.02 ± 2.11	0.967
FE	−0.04 ± 2.11	
BCVA (LogMAR)		
TE	0.44 ± 0.4	<0.001*
FE	−0.01 ± 0.12	
IOP (mmHg)		
TE	15.3 ± 2.6	0.747
FE	15.0 ± 2.3	
OPP (mmHg)		
TE	53.0 ± 7.2	0.94
FE	53.3 ± 6.7	
AL (mm)		
TE	23.5 ± 0.4	0.747
FE	23.7 ± 1.7	
CRT (μm)		
TE	397 ± 120	<0.001*
FE	276 ± 27	

AL = axial length, BCVA = best-corrected visual acuity, Bpm = beats per minute, CRT = central retinal thickness, DBP = diastolic blood pressure, HR = heart rate, IOP = intraocular pressure, MAP = mean arterial pressure, MRSE = manifest refraction spherical equivalent, OPP = ocular perfusion pressure, SBP = systolic blood pressure, SD = standard deviation.
* Marks indicate statistical significance.

Follow-up

Follow-up was performed as fast as possible after intravitreal injection (aim: within 5 min after injection), 30 and 60 min after the injection and included firstly LSFG measurements with simultaneous measurement of blood pressure and heart rate, followed by Goldmann applanation tonometry (GAT).

Statistical analysis

Statistical analysis was performed with SPSS software version 23.0 (SPSS Inc., Chicago, IL, USA). Descriptive data are presented as mean and standard deviation. Data were tested for normality using Shapiro–Wilk test, which confirmed a normal distribution of the data in both groups (treated and FE). Baseline characteristics between treated eyes (TE) and FE were tested for statistically significant differences with Student's *t*-test for unpaired data. Changes in MAP, OPP, IOP and LSFG parameters are given as relative changes, calculated as change in % from baseline (e.g. ΔONH – MA [%] = ((ONH $MA_{after\ injection}$/ONH – $MA_{baseline}$ × 100) − 100)). For interference statistics of the longitudinal data, a general linear model was calculated by repeated-measures analysis of variances (ANOVA). Level of statistical significance was adjusted (Bonferroni correction).

Pearson's correlation was used to determine the relationship between change in IOP or OPP and the observed changes in LSFG parameters.

Results

This study included 20 eyes from 20 patients (45% female) which underwent treatment with IVA in one eye (TE) as well as the 20 FE. The demographics of the patients are shown in Table 1. Best-corrected visual acuity (BCVA) was significantly worse, and

Table 2. Changes in mean arterial pressure (MAP), intraocular pressure (IOP), ocular perfusion pressure (OPP) and mean blur rate at whole optic nerve head region (ONH-MA), at region of big vessels (ONH-MV) and at region of microvasculature (ONH-MT) and at mean blur rate at the choroid (CHOR).

	10 min – Baseline (%; mean ± SD)	30 min – Baseline (%; mean ± SD)	45 min – Baseline (%; mean ± SD)
MAP	9.2 ± 7.4	6.2 ± 13.4	6.8 ± 7.0
IOP			
TE	26.2 ± 35.0	5.1 ± 22.4	0.0 ± 24.3
FE	0.9 ± 18.1	2.7 ± 19.1	−2.9 ± 22.7
OPP			
TE	−4.5 ± 17.8	−0.5 ± 14.9	1.0 ± 14.8
FE	2.3 ± 12.0	−0.7 ± 13.0	1.9 ± 11.7
ONH-MA			
TE	−10.47 ± 9.1	−16.3 ± 9.3	−18.7 ± 10.4
FE	2.0 ± 10.6	−1.5 ± 15.6	−0.6 ± 12.8
ONH-MV			
TE	−14.1 ± 20.4	−23.2 ± 15.1	−27.4 ± 16.5
FE	−5.4 ± 13.1	−7.4 ± 20.5	−9.0 ± 17.7
ONH-MT			
TE	8.1 ± 14.7	6.5 ± 9.2	3.4 ± 12.0
FE	2.4 ± 14.3	−2.7 ± 18.8	−1.5 ± 17.4
CHOR			
TE	−3.8 ± 13.9	3.8 ± 14.0	−4.5 ± 14.4
FE	7.2 ± 10.9	2.9 ± 17.4	−4.0 ± 19.4

Table 3. Repeated-measures analysis of variances results with Bonferroni correction.

	Time	
Measure	p	η^2
MAP	0.756	0.02
IOP		
TE	0.001*	0.294
FE	0.515	0.039
OPP		
TE	0.216	0.077
FE	0.614	0.031
ONH-MA		
TE	<0.001*	0.656
FE	0.555	0.038
ONH-MV		
TE	<0.001*	0.573
FE	0.075	0.133
ONH-MT		
TE	0.042	0.133
FE	0.514	0.041
CHOR		
TE	0.444	0.048
FE	0.017	0.196

η^2 describes the power of the variable.
CHOR = mean blur rate at the choroid, FE = fellow eye, IOP = intraocular pressure, MAP = mean arterial pressure, ONH-MA = mean blur rate at whole optic nerve head region, ONH-MT = mean blur rate at region of microvasculature at optic nerve head, ONH-MV = mean blur rate at region of big vessels, OPP = ocular perfusion pressure, TE = treated eye.
* Marks indicate statistical significance.

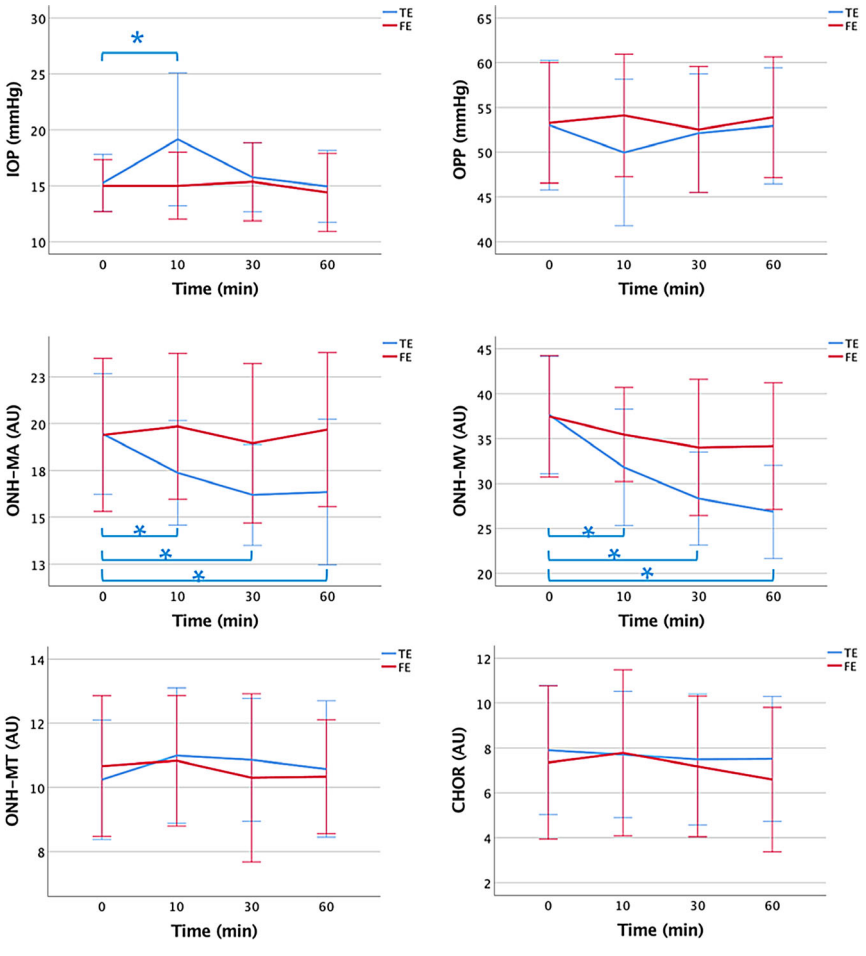

Fig. 1. Line charts of mean and standard deviation indicating changes induced by intravitreal injection in the treated eye (TE) and fellow eye (FE). CHOR = mean blur rate at the choroid, IOP = intraocular pressure, ONH-MA = mean blur rate at whole optic nerve head region, ONH-MT = mean blur rate at region of microvasculature at optic nerve head, ONH-MV = mean blur rate at region of big vessels, OPP = ocular perfusion pressure. Asterisk marks indicate statistical significance for *post hoc* *t*-test with Bonferroni correction between baseline and follow-up measurements within each group.

CRT was significantly larger in the TE group as compared to the FE group.

In all TE, the intravitreal injection was successfully completed. No adverse events were observed during the injection or within the follow-up period. Mean time between injection and first LSFG measurement was 8:56 ± 4:25 min and ranged between 3:38 and 23:06 min.

Table 2 shows the calculated delta values between baseline and follow-up.

A repeated-measures ANOVA with Bonferroni correction was conducted to analyse the effect of the intravitreal injection on the IOP, MAP, OPP and LSFG parameters (Table 3). In the TEs, there was a significant effect on IOP [$F(1.957,37.187) = 7.911$, $p = 0.001$, partial $\eta^2 = 0.294$]; ONH-MA [$F(3,57) = 36.251$, $p < 0.001$, partial $\eta^2 = 0.656$]; ONH-MV [$F(2.214,57) = 25.542$, $p < 0.001$, partial $\eta^2 = 0.573$]; and ONH-MT [F 3,57) = 2.906, $p = 0.042$, partial $\eta^2 = 0.133$]. No significant effects were observed on choroidal LSFG measurements [F (3,54) = 0.907 $p = 0.44$, partial $\eta^2 = 0.048$].

In the FE, no significant alterations in ONH or choroidal perfusion were observed (see Table. 3).

Figure 1 demonstrates changes throughout follow-up of IOP, OPP and ONH-MA, ONH-MV, ONH-MT and CHOR.

Changes in ONH-MA, ONH-MV, ONH-MT and CHOR showed no significant correlation with the change in IOP immediately after injection (ONH-MA $r = -0.182$, $p = 0.443$; ONH-MV $r = -0.65$, $p = 0.786$; ONH-MT $r = 0.096$, $p = 0.688$; CHOR $r = 0.002$, $p = 0.993$).

In 50% (10 eyes) of the injections, no reflux was observed. In six cases, minimal reflux was observed (30%) and in four eyes moderate (20%). The amount of reflux did not correlate with the IOP 15 min after the injection (Pearson's correlation, $p = 0.677$).

Discussion

The present study is the first to evaluate the immediate influence of aflibercept on ocular perfusion in AMD. Our data indicate that IVA rapidly leads to reduced perfusion in the whole ONH (ONH-MA) and the central retinal vessels (ONH-MV) with no significant correlation to the short-term rise in IOP. Intraocular pressure (IOP) increased significantly in the TE after the injection but declined to baseline values after 30 min, whereas MA and MV were significantly reduced up to 60 min after IVA. Alteration of ONH-MT, a parameter for the perfusion of the microvasculature, showed borderline level of significance. An *in vitro* model has shown that aflibercept binds to VEGF with a maximum response after about 100 seconds (Sivertsen

et al. 2018); thus, our results could indicate a direct pharmacological effect on the perfusion independent of the IOP increase. Previous studies using colour Doppler imaging on eyes with AMD showed that the retrobulbar circulation was decreased 1 week after a bevacizumab injection (Toklu et al. 2011). More recently, this was also shown for intravitreal aflibercept (Gok & Kapti 2018). Lately, another paper reported significant vasoconstriction of the retinal arterioles after three monthly injections of aflibercept using a computer-based approach (Tetikoğlu et al. 2018).

Laser speckle flowgraphy (LSFG) data on ocular perfusion after anti-VEGF treatment were published for patients with macular oedema after branch retinal vein occlusion (BRVOME) or diabetic macular oedema (DME). Intravitreal injection of bevacizumab significantly decreased the blood flow in the ONH, retinal artery and vein and the CHOR in patients with DME 1 week and 1 month after injection (Kunikata et al. 2014).

Optic nerve head (ONH)-MA, ONH-MV and ONH-MT in the FE maintained stable throughout the whole follow-up of our protocol, indicating that aflibercept does not enter the systemic circulation, at least not within the first hour after intravitreal injection. This result is in agreement with results published on ranibizumab in patients with DME or BRVOME (Sugimoto et al. 2017).

Choroidal perfusion was maintained stable in both, the treated and the FE up to 1 hr after the injection. Studies on the blood flow autoregulation in the human CHOR have been published before. In agreement with these previous studies, our data indicate that the CHOR has some autoregulatory capacity in response to small changes in IOP (Riva et al. 1997; Simader et al. 2009; Schmidl et al. 2012). It has been also shown that choroidal autoregulation depends on pressure levels at both the arterial and the venous sides, indicating its complexity (Longo et al. 2004; Schmidl et al. 2016). In patients with DME, it has been shown that the choroidal MBR decreases significantly 1 week and 1 month after injecting bevacizumab (Kunikata et al. 2014). Whether this is related to the breakdown of autoregulation in patients with diabetes remains to be shown (Movaffaghy et al. 2002). However, choroidal thickness has lately been shown to decline in patients with AMD following three injections of aflibercept (Tetikoğlu et al. 2018). As expected, we observed transient rise in IOP, but no correlation with the amount of reflux was shown. It has been previously speculated that reflux led to decreased IOP rise; however, other studies did not quantify reflux (Sharei et al. 2010; Lemos-Reis et al. 2014).

Our study was limited by a small sample size. Also, due to logistic factors as well as patient factors, in many cases we did not manage to perform the LSFG measurements within 5 min after the injection. However, statistical results indicate significant differences with high power.

In conclusion, this study adds information about the short-term effects of aflibercept on ocular perfusion. Results indicate that aflibercept has an immediate direct and significant effect on the retinal perfusion of the TE but not on that of the FE. Choroidal blood flow on the other side is not significantly altered in the treated or in the FE up to 1 hr after IVA.

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