Effects of three intravitreal injections of aflibercept on the ocular circulation in eyes with age-related maculopathy

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ABSTRACT

Aims To investigate changes in ocular perfusion following three consecutive intravitreal injections with aflibercept for treatment of neovascular age-related macular degeneration (nAMD).

Methods The study included 20 eyes from 20 Caucasian patients with unilateral nAMD and 20 fellow eyes. All nAMD eyes were treated with standard intravitreal injection of aflibercept (IVA; 2 mg). Measurements of ocular perfusion at the optic nerve head (ONH) and the choroid were performed with laser speckle flowgraphy (LSFG). Measurements were conducted at baseline, 1 week after the first injection, at the time point of the second and third injection as well as 1 month after the third injection.

Results In treated eyes, mean blur rate (the main output parameter of LSFG) in the ONH microvasculature and in the choroid was significantly reduced 1 week after the first IVA treatment. The effect persisted throughout the entire follow-up period ($p<0.001$). No change in ocular perfusion was observed in fellow eyes.

Conclusions IVA for treatment of nAMD leads to a reduction in perfusion of the ONH and the choroid in the treated eye with no apparent effect on the fellow eye.

INTRODUCTION

Age-related macular degeneration (AMD) is one of the main causes of irreversible visual loss worldwide.[1] Neovascular AMD (nAMD) is characterised by choroidal neovascularisation (CNV).[2] Vascular endothelial growth factor (VEGF) is crucial for the development of CNV.[3] The therapy with intravitreal injections of anti-VEGF molecules has evolved to be the first-line treatment for nAMD. There are several types of commercially available VEGF antagonists, the older representing monoclonal antibodies (ranibizumab and bevacizumab), whereas aflibercept, a newer substance, is a receptor fragment. Both ranibizumab and bevacizumab inhibit solely VEGF-A.[4 5] In contrast, aflibercept is a recombinant fusion protein that inhibits VEGF-A, VEGF-B and placental growth factor.[6]

On a physiological level, VEGF acts as a vasodilator as it activates endothelial nitric oxide synthase (eNOS), which produces the potent vasodilator nitric oxide (NO).[3]

Hence, it has been hypothesised that therapeutic VEGF inhibition induces vasoconstriction in retinal and optic nerve head (ONH) vessels with a subsequent decrease in ocular perfusion.[7] A range of studies have indicated that both bevacizumab[7 8] and ranibizumab[9 10] induce alterations in ocular perfusion with sustained vasoconstrictive effects reported for up to 1 year after administration of the drug.[10] Aflibercept exhibits an almost 100 times greater binding affinity for VEGF compared with bevacizumab and ranibizumab as well as substantially prolonged binding activity.[11] While the pharmacokinetic properties of aflibercept may be beneficial in the treatment of AMD-associated CNV lesions, they may also act as a drawback with regard to potentially enhanced vasoconstrictive effects on the vasculature of the retina and optic nerve and therefore on ocular perfusion.

Various approaches to measure ocular perfusion have been described in the literature.[12–15] For the purpose of this study, laser speckle flowgraphy (LSFG) was employed. It enables two-dimensional, non-invasive measurements of perfusion at the ONH, the retina and the choroid using the laser speckle phenomenon.[16] LSFG has been shown to exhibit excellent repeatability in Caucasian subjects,[13] and the technology has been used to assess the influence of intravitreal injection of bevacicumab and ranibizumab on ocular perfusion in patients with diabetic retinopathy, retinal vein occlusion or central serous chorioretinopathy.[8 10 17–19] To our knowledge, this is the first study to investigate the effect of intravitreal aflibercept on ocular perfusion.

MATERIALS AND METHODS

Patients

This study included 20 eyes from 20 patients (45% female; mean age 75.1±8.2 years) who underwent treatment with intravitreal injection of aflibercept (IVA) in one eye (treated eye (TE)) as well as the 20 fellow eyes (FE). The protocol adhered to the guidelines of the Declaration of Helsinki.

Written informed consent was obtained before inclusion in the study. The inclusion criteria were (1) age >50 years and (2) patients scheduled for three consecutive IVA (4-week intervals) 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) vitrectomised eyes, (4) ametropia >6 diopter and (5) any relevant ophthalmic diseases/conditions that could interfere with LSFG measurements (eg, opacities of the

optical media, glaucoma, ONH drusen, tilted disc, etc). Pretreatment with intravitreal anti-VEGF was not an exclusion criterion, but last treatment must have been received at least 3 months prior to study inclusion.

Study protocol

At baseline, all subjects underwent a thorough ophthalmic evaluation. Assessments comprised slit-lamp examination with indirect funduscopy and measurement of intraocular pressure (IOP) using Goldmann applanation tonometry. Best-corrected visual acuity (BCVA) was tested with the standard ETDRS visual acuity chart. Optical coherence tomography(OCT)(Spectralis, Heidelberg Engineering, Heidelberg, Germany) was employed to measure central retinal thickness (CRT), defined as the thickness from Bruch membrane to internal limiting membrane in a circle with 1 mm diameter centered at the fovea. Scanning protocol was macular volume scan including 25 line B-scans at a dimension of 20°×20°.

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.[20]

Laser speckle flowgraphy

LSFG measurements were performed with the LSFG RetFlow (Nidek Co., LTD, Gamagori, Aichi, Japan) with dilated pupil (0.5% tropicamide eye-drops; Mydriaticum Agepha Augentropfen; Agepha Ges.m.b.H., Vienna, Austria). The technical principles of LSFG have been described elsewhere.[21] The main output parameter of LSFG, mean blur rate (MBR), describes the blurring of the pattern of speckle contrast produced by the interference of a coherent light (eg, a laser) scattered by moving blood cells in the back of the eye. MBR was calculated for the total ONH area, defined by an ellipsoid region of interest (ROI) that is referred to as ONH-MA ('MBR of all area'). By using the on-board software, the MBR was then calculated in areas of the large retinal vessels within the ONH (ONH-MV, 'MBR of vascular area') as well as in the area of the ONH containing the microvasculature (ONH-MT, 'MBR of tissue area'). For analysis of the choroid (CHOR), a square ROI was set (150×150 pixels) in a temporal location one disc diameter away from the disc margin without including the main retinal vessels as it was described recently (see figure 1).[22] All ROI positions and dimensions were saved and remained unchanged in the follow-up measurements. All study participants were instructed to abstain from alcohol and stimulating beverages containing xanthine derivatives (eg, tea, coffee) 12 hours before the LSFG measurements to avoid any bias.[23]

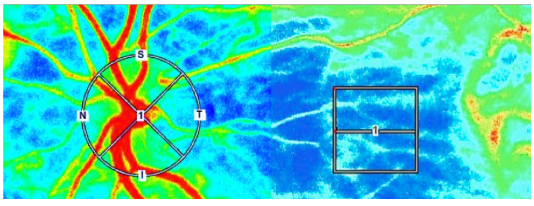

Figure 1 Representative composite colour map image from laser speckle flowgraphy measurements. For blood flow analysis, an ellipsoid rubberband was set around the optic nerve head. For analysis of the choroid, a square rubberband was set in a temporal location one disc diameter away from the disc margin.

Intervention

All patients were scheduled for three consecutive monthly IVA (Eylea, Bayer, Berlin, Germany). All injections were performed in the operating theatre following the standard aseptic intravitreal technique. After instillation of topical anaesthetic (0.4% oxybuprocaine hydrochloride and lidocain; produced by institutional pharmacy), sterilisation 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.

Follow-up

Baseline measurement was performed immediately before the first injection of aflibercept. Patients were re-evaluated 1 week after the first injection, before the second and third IVA and 1 month after the last IVA. At each follow-up visit, patients were evaluated with LSFG with simultaneous measurement of blood pressure and heart rate as well as IOP measurement and OCT acquisition. Outcome measures included the change in CRT and the perfusion of larger retinal vessels, the ONH and the choroid.

Statistical analysis

Statistical analysis was performed with SPSS software V.23.0 as intention to treat. Descriptive data are presented as mean and SD. Data were tested for normality using Shapiro-Wilk test, which confirmed a normal distribution of data in both groups (TEs and FEs). Baseline characteristics between TEs and FEs were tested for statistically significant differences with the Student's t-test for unpaired data. Changes in MAP, OPP, IOP as well as LSFG parameters are given as relative changes, calculated as change in percentage from baseline (eg, 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 for repeated measurements (Bonferroni correction).

RESULTS

Demographics at baseline (before injection) can be found in the table 1. One subject developed exudative AMD in the FE after the third IVA and was therefore excluded before the last visit. One subject was lost to follow-up after the third IVA.

In all subjects, LSFG measurements could be successfully obtained. In the TEs, a statistically significant decrease in CRT from 389±97 µm at baseline to 271±63 µm 4 weeks after the third injection was observed with spectral domain-OCT (p<0.001). In the TEs, visual acuity tended to increase from 64 ETDRS letters to 67 (p=0.093) at the last visit 1 month after the third IVA. In the FEs, visual acuity (84 ETDRS letters respective 86, p=0.126) and CRT (278±32 µm respective 280±32 µm, p=0.163) were stable between baseline and last follow-up.

With respect to alterations in ocular perfusion, absolute changes of ONH-MA, ONH-MV, ONH-MT and CHOR are illustrated in figure 2. The figure 3 gives representative colour map images of the LSFG measurements of a patient in this study. The table 2 shows the relative delta values between baseline and follow-up of the ocular perfusion.

A repeated measures ANOVA with Bonferroni correction (level of statistical significance ≤0.007) was conducted to analyse the effect of IVA on MAP, IOP, OPP and the respective LSFG-derived parameters. In the TEs, there was a significant effect on

Table 1 Baseline demographics of the study population including results of Student's t-test for unpaired data comparing means of TEs and FEs

Characteristics		Number, mean±SD	P value
Sex	Male	11	
	Female	9	
Age (years)		75.1±8.2	
SBP (mm Hg)		143.1±18.8	
DBP (mm Hg)		82.1±9.3	
HR (beats per minute)		71.7±9.8	
MAP (mm Hg)		102.4±10.6	
MRSE (diopter)	TE	−0.02±2.11	0.967
	FE	−0.04±2.11	
ETDRS letters	TE	63±20	<0.001*
	FE	84±8	
IOP (mm Hg)	TE	15.3±2.6	0.747
	FE	15.0±2.3	
OPP (mm Hg)	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	
Number of previous injections	TE	6±5	<0.001*
	FE	0.3±1	

CRT, central retinal thickness; DBP, diastolic blood pressure; FE, fellow eyes; HR, heart rate; IOP, intraocular pressure; MAP, mean arterial pressure; MRSE, mean refractive spherical equivalent; OPP, ocular perfusion pressure; SBP, systolic blood pressure; TE, treated eyes.

ONH-MT ($F(4, 64)=7.326$, $p<0.001$, partial $\eta^2=0.314$) and CHOR ($F(2.132, 34.108)=9.488$, $p<0.001$, partial $\eta^2=0.372$); ONH-MA only reached borderline level of significance ($F(1.859, 29.74)=4.285$, $p=0.687$, partial $\eta^2=0.021$).

In the FEs, no significant alterations in ONH or choroidal perfusion were observed. MAP as well as IOP and OPP were stable (in TE and FE) throughout the entire follow-up (see table 3).

DISCUSSION

This study investigated the mid-term effects of aflibercept on ocular perfusion in unilateral nAMD. IVA leads to significantly reduced perfusion in the ONH-MT and the CHOR as measured with LSFG as early as 1 week after the injection. The effect was observed up to 1 month after the third monthly IVA and was not seen in FEs.

We consider our results in accordance with other published studies on intravitreally administered anti-VEGF agents. A trial from 2011 investigated the influence of 3 monthly bevacizumab injections on ocular blood flow in nAMD.[7] The authors analysed short-term and long-term effects of the anti-VEGF agent by means of a non-invasive bidirectional laser Doppler velocimeter that directly measures the Doppler shift of the backscattered light from the red blood cells. In addition, they used scanning laser Doppler flowmetry (SLDF) to measure tissue perfusion at the neuroretinal rim as well as the peripapillary retinal blood flow. It was shown that retinal arteriolar diameter decreased significantly 1 week after the first intravitreal injection of bevacizumab, and this decrease persisted until the end of the study (5 weeks after the third injection), suggesting a long-term effect of bevacizumab on vascular tone. However, comparable to our

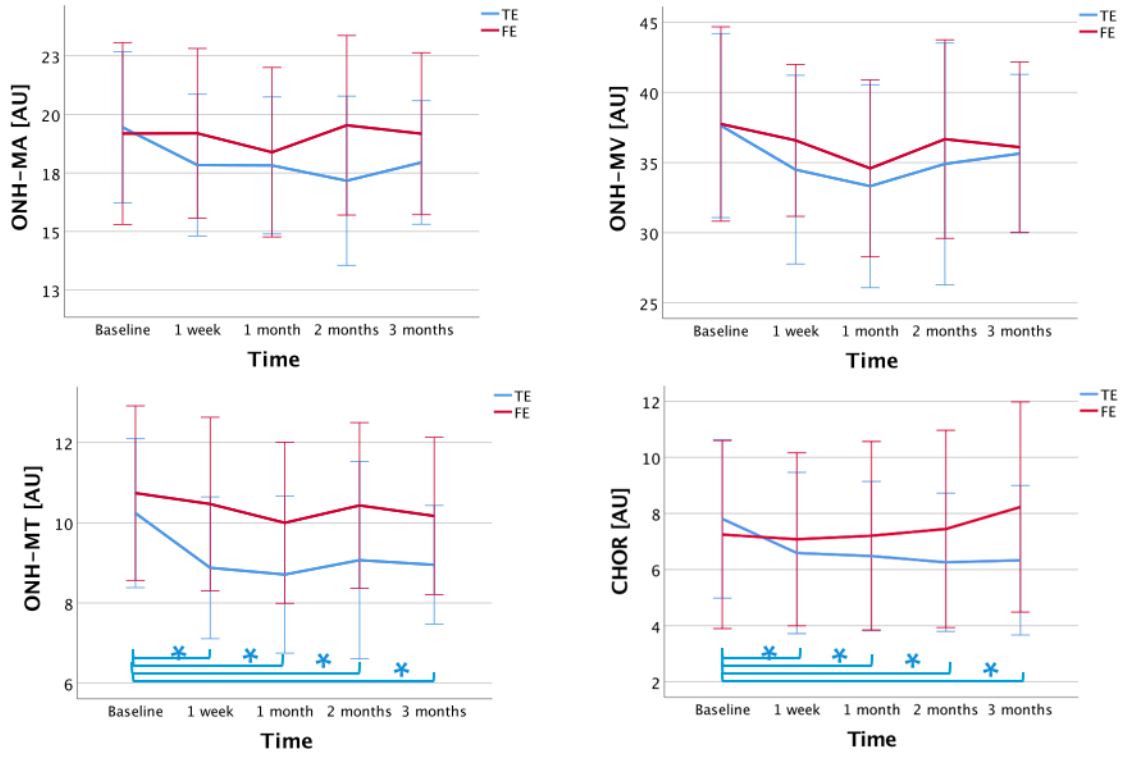

Figure 2 Line charts of mean and SD indicating changes induced by intravitreal injection in the treated eye (TE) and fellow eye (FE). Asterisk marks statistical significance for post hoc t-test with Bonferroni correction between baseline and follow-up measurements within each group. CHOR, mean blur rate at the choroid; ONH, optic nerve head; ONH-MA, mean blur rate at whole ONH region; ONH-MV, mean blur rate at region of big vessels within the ONH; ONH-MT, mean blur rate at region of microvasculature at ONH.

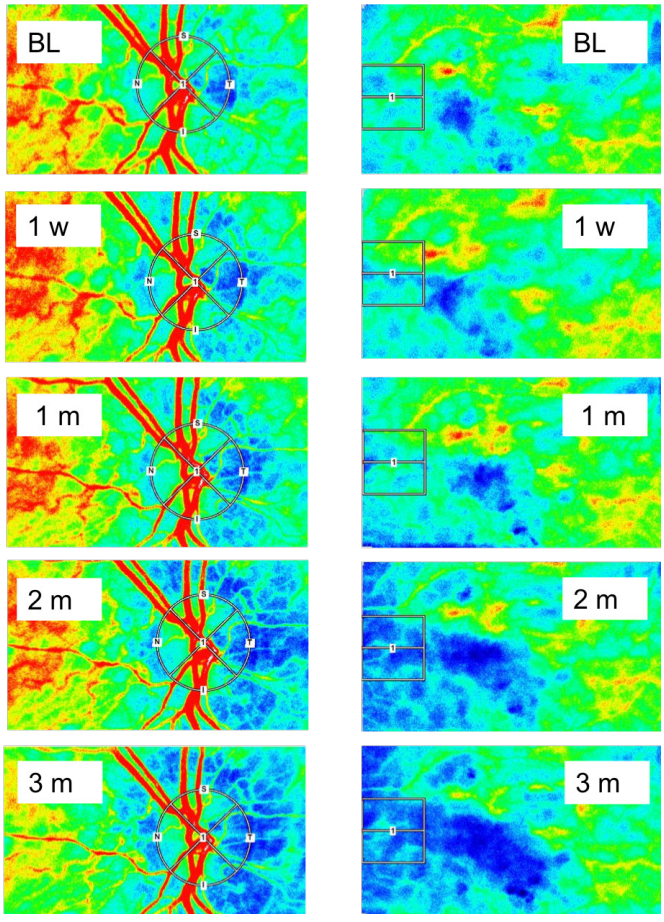

Figure 3 Representative colour map images from laser speckle flowgraphy measurements of a patient from this study. Measurements centred at the optic nerve head on the left side, measurements centred at the fovea on the right side. BL, baseline; 1 w, measurement 1 week after first injection; 1 m, measurement before second injection; 2 m, measurement before third injection; 3 m, measurement 1 month after third injection.

results, they observed no significant velocity change at any visit. They observed a slight continuous decrease in arteriolar blood flow which did not reach statistical significance until 5 weeks after the third injection of bevacizumab. The blood flow at the optic disc rim area decreased with borderline significance. Peripapillary retinal blood flow did not change significantly.[7] The authors interpret the decrease in rim perfusion observed with SLDF as reduced deeper circulation, coming from the short posterior ciliary arteries, in response to bevacizumab.[7]

Other studies have been published analysing the effects of anti-VEGF on ocular perfusion in various retinal vascular diseases. It has been shown with LSFG that the intravitreal injection of bevacizumab significantly decreased the blood flow in the ONH, retinal artery and vein and the choroid in patients with diabetic macular oedema, 1 week and 1 month after injection.[8] Bevacizumab was previously shown to reduce fenestration of the normal choriocapillaris.[24] Hypothesising, aflibercept has the same effect on the choriocapillaris, this could explain the observed alteration in choroidal blood flow. For ranibizumab, the effect on the choroidal perfusion has been shown to be dependent on prior panretinal laser coagulation.[25]

We could not observe a significant effect of IVA on the FEs in any of the MBR parameters. This is in accordance with the results published on the effect of ranibizumab on the untreated FEs in eyes with diabetic macular oedema or macular oedema after branch vein occlusion.[10]

Under physiological conditions, VEGF acts as a vasodilatator by activating endothelial nitric oxide synthase.[26] In an in vitro mouse model, it was found that VEGF is necessary for the maintenance of the choriocapillaris.[27] The development of geographical atrophy (GA) during treatment with anti-VEGF for nAMD has been analysed in several clinical trials with conversion rates described between 3% and 18% after 2 years.[28 29] Our results confirm the effect of anti-VEGF on the choroidal perfusion, which might be associated with the potential risk of developing GA.

Our study was limited by a small sample size. The majority of the patients had received anti-VEGF treatment before inclusion in the trial. It has been discussed before that the gain in visual acuity is mostly pronounced in the first years of therapy and stagnates afterwards.[30 31] In addition, the protocol did not provide a

Table 2 Changes of ONH-MA, ONH-MV and ONH-MT as well as CHOR

		1 week versus BL (%) (mean±SD)	1 month versus BL (%) (mean±SD)	2 months versus BL (%) (mean±SD)	3 months versus BL (%) (mean±SD)
ONH-MA	TE	−8.5±6.9	−7.9±8.6	−11.7±17.3	−8.5±6.6
	FE	1.0±27.1	−1.2±25.2	5.8±28.1	3.2±27.3
ONH-MV	TE	−9.3±7.8	−11.1±4.6	−8.8±19.0	−6.5±11.8
	FE	−3.8±17.2	−6.6±18.0	1.1±21.6	−2.8±22.6
ONH-MT	TE	−12.7±11.0	−14.9±10.0	−12.1±16.1	−12.9±7.1
	FE	0.1±28.7	−3.8±25.1	1.1±28.5	−1.1±31.4
CHOR	TE	−17.2±16.7	−17.6±12.2	−18.6±12.0	−16.7±12.6
	FE	0.2±17.1	−0.6±9.9	4.0±11.9	9.8±25.1

BL, baseline; CHOR, mean blur rate at the choroid; FE, fellow eye; ONH, optic nerve head; ONH-MA, mean blur rate at whole ONH region; ONH-MT, mean blur rate at region of microvasculature at ONH; ONH-MV, mean blur rate at region of big vessels within the ONH; TE, treated eye.

Table 3 Repeated measures analysis of variances results with Bonferroni correction (level of statistical significance ≤0.007)

		Time	
Measure		P value	η^2
MAP		0.115	0.108
IOP	TE	0.586	0.043
	FE	0.401	0.06
OPP	TE	0.123	0.106
	FE	0.126	0.105
ONH-MA	TE	0.025	0.211
	FE	0.687	0.021
ONH-MV	TE	0.136	0.120
	FE	0.169	0.1
ONH-MT	TE	<0.001*	0.314
	FE	0.341	0.068
CHOR	TE	<0.001*	0.372
	FE	0.151	0.098

(η^2) describes the power of the variable.
*Statistical significance.
CHOR, mean blur rate at the choroid; IOP, intraocular pressure; MAP, mean arterial pressure; ONH, optic nerve head; ONH-MA, mean blur rate at whole ONH region; ONH-MT, mean blur rate at region of microvasculature at ONH; ONH-MV, mean blur rate at region of big vessels within ONH; OPP, ocular perfusion pressure.

control group receiving intravitreal injections of solely buffered saline solution or comparable fluids because of ethical reasons. It seems very unlikely that the injection process itself has a long-term effect on the ocular perfusion. However, this possibility cannot be entirely excluded.[7] LSFG itself has a notable limitation as it is incapable of producing absolute values. However, it is an acceptable approach to monitor intraindividual changes in perfusion over time.[32]

In conclusion, the results indicate that aflibercept may elicit a prolonged reduction of perfusion of the ONH and the choroid of the TE. This could result in the development of GA. Our findings warrant further investigation of potentially negative effects of anti-VEGF treatment on ocular circulation in general, particularly in vulnerable (eg, glaucomatous) eyes. However, the beneficial effect of aflibercept on the CNV is not to be discussed and the treatment with VEGF antagonists remain the standard of care in exudative AMD.

Contributors ASME provided data acquisition and analysis, original draft, interpretation, oversight and final draft. NL provided original concept, oversight and final version. DP provided data acquisition. LS provided critical revising, oversight and final version. MB contributed in critical revising and final approval.

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