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GLAUCOMA

Dynamics of retinal vessel response to flicker light in glaucoma patients and ocular hypertensives

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Abstract
Purpose To analyze dynamics of retinal vessel dilation response to flicker light in patients with glaucoma and ocular hypertension.
Patients and methods Response to flicker light was measured in retinal vessels by means of Retinal Vessel Analyzer. After the baseline 50 seconds long diameter recording of inferior and superior temporal artery and vein, three flicker stimulations of 20 seconds duration was applied, with a 80 seconds break in between. Area under the curve of the vessel diameter (AUC) was compared during 3 flicker periods in the open angle glaucoma patients group (POAG, n=47) and ocular hypertensives (OHT, n=46) and age-matched healthy controls (n=56)
Results POAG eyes demonstrated smaller response of all vessels to flicker light in general than the other two groups (p=0.0008), but the response dynamics was significantly different between the groups (p=0.038), showing in three flicker periods a delayed increasing response in the POAG and OHT groups, and remaining stable in healthy subjects.
Conclusion General vessel response to flicker light was decreased in POAG patients despite the slow improvement in repeated flicker stimulation, indicating an altered response pattern.

Keywords Retinal vessels · Flicker · Glaucoma · Ocular hypertension

Clinical trial registration ClinicalTrials.gov NCT00430209

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Introduction

Glaucoma is an optic neuropathy characterized by morphologically typical changes in the optic nerve head and accompanied by typical defects in the visual field. Apart from intraocular pressure, blood flow disturbances are believed to represent a major contributor to glaucomatous damage [1]. The term neurovascular coupling refers to a rapid response of neural tissue blood vessels to increased neuronal activity, and it has recently been investigated in glaucoma patients and patients with ocular hypertension [2, 3]. Optical accessibility of the retinal vessels and the light sensitivity of the retinal neural circuitry make the use of the Retinal Vessel Analyzer (RVA; IMEDOS GmbH, Weimar, Germany) particularly appealing in this field of research [4–6]. Recently, specific protocols for the use of this device have been discussed and published [7], as well as data on technical (measurement error) and biological (variability of vessel response to flickering light) reproducibility [8, 9]. Neurovascular coupling makes it possible to meet an increased perfusion demand of the neuronal tissue on a very short time scale [10, 11]. Ischemia–reperfusion damage is postulated as one of the important mechanisms leading to glaucomatous optic neuropathy [12]. Increased short-term variability of both the blood pressure and local ocular choroidal perfusion in glaucoma patients [13] seems to support this hypothesis. Disturbances in the neurovascular coupling could represent an additional risk factor for glaucoma, as they could lead to underperfusion of retinal ganglion cells in times of critical need. We investigated a retinal vessel response to flicker light in untreated patients with glaucoma and ocular hypertensives. In the present analysis, we focus on the dynamics of this response to three consecutive repeated flicker-light stimulations.

Patients and methods

Patients

Consecutive primary open-angle glaucoma (POAG) patients and patients with ocular hypertension (OHT) were recruited for the study from the glaucoma consultations at the University Eye Clinic Basel. Tenets of the Declaration of Helsinki were followed. After approval by the ethics committee, we obtained informed consent from our subjects. The study was registered at ClinicalTrials.gov and the recruitment was conducted between 2007 and 2010. Newly diagnosed and therapy-naive POAG and OHT patients meeting study criteria underwent study examinations; patients on IOP-lowering therapy were first subjected to a 4-week wash-out phase. Healthy controls were recruited through ads in local newspapers. Subjects were screened for ocular and systemic diseases. A detailed medical and ophthalmic history was recorded, and all subjects completed an ophthalmological examination, including IOP daily profiling with Goldmann applanation tonometry (IOP readings were taken at 8 h, 11 h and 16 h), and ultrasound pachymetry. POAG was diagnosed based on glaucomatous optic disc cupping (in particular: thinning of the inferior and/or superior rim, cup-to-disc ratio asymmetry of >0.2), and based on matching glaucomatous visual field defects [14]. Native IOP was neither exclusion nor inclusion criterion for POAG diagnosis; hence, patients were included from both the "high tension" and "normal tension" side of the IOP spectrum in POAG. In contrast, at least two daily readings of naive IOP of equal or above 21 mmHg, in absence of disc or visual field damage, were required for the diagnosis of OHT. All participants with diabetes mellitus, untreated or unstable essential hypertension, untreated or unstable hypercholesterolemia, drug or alcohol abuse, history of eye surgery except pseudophakia, high ametropia (spherical equivalent<−5 diopters or>+3 diopters), astigmatism above 2 diopters, significant cataract, pigment or PEX dispersion syndrome, history of an acute glaucoma attack, and any form of secondary glaucoma were excluded from the study. Smoking was an exclusion criterion [15]. In the POAG group, an eye with the most advanced damage (higher mean VF defect and thinner peripapillary RNFL as demonstrated by the optical coherence tomography), in the OHT group an eye with the higher mean diurnal IOP was selected, and in healthy controls one randomly selected eye per subject entered the analysis.

Methods — retinal vessel analyzer

Details on the device and its application have been published previously [4, 5, 7, 16]. In short, this device consists of a fundus camera (FF450, Zeiss Jena, Germany) for the examination and recording of the ocular fundus, and the RVA control computer which allows continuous as well as offline readings of the retinal vessel diameter. An optoelectronic shutter is inserted in the fundus camera to interrupt the observation light (530–600 nm, irradiance at the fundus approximately 1.96×10–4 W/cm2) over the entire 30° visual field of the retinal camera. This provides a bright-to-dark contrast of 25:1 and at standard European video frequency of 25 Hz produces a sequence of one normal illuminated and one dark single frame at the arbitrarily chosen (or more precisely said, chosen for technical reasons) shutter frequency of 12.5 Hz of rectangular light interruption. This frequency, however, does lie in the range of the maximally exciting flicker frequency [17, 18].

Four major temporal vessel segments, one vein and one artery, one of each inferior and superior, was selected for our measurements. The exact location of the measurement was governed by the geometry of the vessel segments. Three cycles of 20 seconds flicker light followed by 80 seconds of still illumination were conducted after an initial, still measurement period of 50 seconds. The length of the chosen vessel segment was again determined by the vessel geometry, but was in all cases between 500 and 750 micrometers, which corresponds to 40 to 60 measured diameter values (the device is set to obtain one transverse diameter reading, based on vessel image brightness profile, at each 12.5 micrometer vessel length). Stability of vessel location where diameter, measured in an automated sequence described above, is ensured by the eye/image-tracking software, which is in turn based on the vessel branching geometry.

Experimental procedure and data analysis

Pupil was dilated with tropicamide drops in all subjects. After a minimum of 10–15 minutes in still seating position, a blood pressure reading was taken, and the RVA measurement conducted in the selected eye. RVA measurements were all conducted by the same experienced researcher (AK). For the analysis reported in this paper, an average vessel diameter was calculated for the whole chosen vessel segment, and its behaviour analyzed on the time scale. Area under the curve (AUC) was calculated for the baseline 50-second measurement, providing in fact an average baseline diameter of the given vessel, and then the AUC of vessel diameter during each of the 20-second-long flicker periods (in fact, the mean vessel diameter during the 20-second flicker). The latter three values were then expressed as a percentage change to the corresponding baseline diameter, in other words they were standardized with the respective baseline value. One-way repeated measures analysis of variance was performed, one-way ("independent") factor being the three experimental groups,

and "repeated" factors being: (I) the three repeated readings under the flicker light stimulation, (II) arteries/veins, and (III) inferior/superior temporal vessels. In essence, this was a four-way ANOVA with one independent and three "dependent/repeated" measures. Of particular interest was the interaction between the factor "group" and the three repeated measurements, with the intention to describe the dynamics of the retinal vessel response to repeated flicker-light stimulation in three different groups. An intraocular pressure reading was taken immediately after the RVA measurement.

Results

The original study goal was set at 50 subjects for each group. In order to have a safety margin, because poor fixators and patients with poor RVA data quality were to be excluded, recruitment was continued in the control group and the POAG group above this threshold. OHT patients meeting the criteria represented a recruitment challenge, and the recruitment was stopped as soon as the number of 50 was reached. In the ultimate analysis, however, one OHT patient had to be excluded due to poor data quality. All together, 59 control subjects (59±9 years of age), 55 POAG patients (60±10), and 49 OHT patients (62±9), entered the analysis. Intraocular and blood pressure values, as well as central corneal thickness and average baseline vessel diameters, are presented in Table 1.

With regard to the potentially vasoactive systemic therapy at the moment of study examinations, treatment was as follows:

- In the POAG group: 23 patients had no therapy, six had statines, three beta-blockers, five diuretics, seven ACE blockers, two calcium channel blockers, five magnesium, five aspirin, four had antidepressants (some patients had more than one of the mentioned medications)
- In the OHT group: 27 patients had no therapy, eight had statines, five beta-blockers, three diuretics, three ACE blockers, four magnesium, seven aspirin, five had antidepressants, one had hormonal therapy (some patients had more than one of the mentioned medications)
- In the healthy control group: 37 patients had no therapy, three had statines, one beta-blocker, one diuretic, four ACE blockers, three magnesium, two aspirin, two had antidepressants, five had hormonal therapy (some patients had more than one of the mentioned medications).

The Chi-square test, analyzing the number of therapy-free patients vs patients with therapy, was between each pair of groups not significant (smallest $p=0.08$).

The ANOVA model comprised the following factors: three experimental groups (controls, POAG and OHT patients), arteries/veins, inferior/superior temporal vessels and AUC values (change to baseline in percentage) during three flicker periods of 20 seconds. Results were the following. The factor "group" had a p-value of $p=0.0008$ (controls 2.24±1.76 %, OHT 1.98±1.85 %, POAG 1.63± 1.57 %). Planned comparisons were made between controls and POAG patients and they were found significantly different ($p<0.001$), between OHT and POAG patients as well ($p=0.038$), whereas controls and OHT patients had a statistically comparable overall mean vessel response to flicker ($p>0.1$). The factor "arteries/veins" had a p-value of $p=0.83$, indicating a similar overall response of arteries and veins (1.96% vs 1.93% respectively; reminder: values are expressed in percentage change to average baseline value). The factor "inferior vs superior" had a p-value of $p<0.001$, indicating a stronger vessel response inferior than superior (overall means 2.25% vs 1.65%). This difference between

Table 1 Parameters of interest at baseline, mean ± SD (units in brackets), with results of the one-way analysis of variance (ANOVA) and post-hoc Tukey honest significant difference test between groups, where appropriate

Parameter	Healthy controls (1)	POAG patients (2)	OHT patients (3)	Statistics (ANOVA)
Gender (m/f)	32/27	23/2	26/23	
Average retinal arterial diameter (micrometers)	113±14	110±13	111±15	$p=0.33$
Average retinal venous diameter (micrometers)	143±20	140±19	142±19	$p=0.49$
Naive intraocular pressure (mmHg)	13.6±2.8	16.9±4.5	21.4±4.0	$p<0.001$ (all post–hoc Tukey HSD test pairs significant)
Central corneal thickness (micrometers)	545±38	539±51	562±37	$p<0.001$ (in post–hoc Tukey HSD test, OHT different to the other two)
Systolic blood pressure (mmHg)	141±22	134±19	147±26	$p=0.07$
Diastolic blood pressure (mmHg)	81±12	76±11	80±13	$p=0.06$

inferior and superior temporal retinal segments was comparably present in arteries and veins (interaction between factors "arteries/veins" and "inferior/superior" was $p=0.53$).

With all groups taken together, the fourth factor (three repeated flicker stimulations) demonstrated a p-value of $p=0.02$, with overall response means of 1.8% vs 2.0% vs 2.1%. When, however, the groups were analyzed separately in this regard, by means of interaction between the factor "group" and the factor "repeated flicker measurements", p-value was $p=0.04$, indicating a response increase in subsequent flicker stimulation in the OHT and POAG group (no significant difference between the two, planned comparisons, $p=0.61$), as opposed to constant and stable vessel response in the control group throughout three flicker periods (planned comparisons, POAG vs controls $p=0.03$, OHT vs controls $p=0.007$).

Figure 1 represents the AUC in three groups during three flicker periods; inferior and superior, as well as arteries and veins, are collapsed together in order to graphically depict the main finding of this analysis — differences in the dynamics of the vessel response across groups.

Discussion

In the present study, we focus on the results of the dynamics analysis of the retinal vessel response to repeated light stimulation in POAG and OHT patients and age-matched controls. On average, all retinal vessels of the

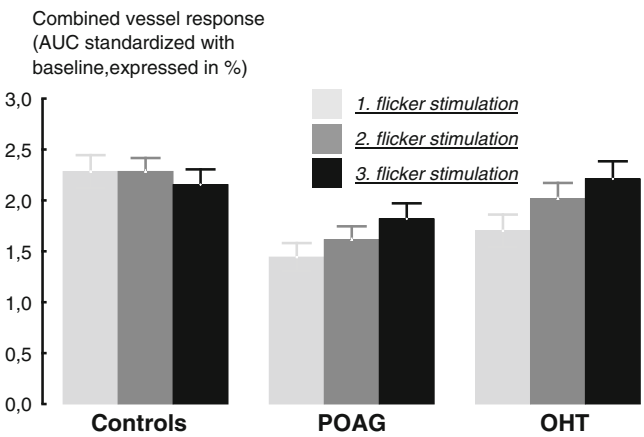

Fig. 1 All measured retinal vessels (arteries and veins, inferior and superior temporal vessels) pooled together. Depicted parameter (mean ± SE) is the area under the curve (AUC) during each 20 seconds of flicker stimulation, standardized with baseline vessel diameter level. AUC is in fact the mean vessel diameter during the 20 seconds of flicker stimulation; this value was expressed as change to baseline in percentages (%) of baseline, and hence represents a mean vessel diameter increase during the flicker stimulation. Significant differences of this parameter across groups and the three flicker periods are reported in detail in the text of the Results section

POAG patients, arteries as well as veins, demonstrated a diminished response to flicker light stimulation. This mean response was clearly smaller than in controls, and borderline smaller than in OHT patients. There was no significant difference in the mean vessel response between the OHT patients and controls; however, the dynamics of this response during three flicker periods was different. The response dynamics was comparable between the OHT and the POAG group, and both demonstrated significantly different behaviour of the response compared to controls.

Garhofer et al. [2] found significantly diminished retinal vein response to flickering light in patients with early glaucoma compared with healthy volunteers. Results of the present study are similar, except arteries seem to demonstrate an altered response as well. Direct comparison of the vessel response dynamics with the above study is not possible, as a different flicker protocol was used.

Based on the present data, it remains an open question whether generally diminished response of all retinal vessel in POAG patients is a cause or a consequence of the glaucomatous damage. If the former, a vascular dysregulation compromising the neurovascular coupling response might be present in glaucoma patients, and might have contributed to the glaucomatous damage [6]. If the latter, less tissue, as a result of glaucomatous damage, could mean less perfusion demand, and hence on the average smaller retinal vasodilation — in contrast to OHT patients, the vessel response level of the POAG remained below that of controls in all three flicker repetitions.

In contrast to controls, where the vessel response was stable throughout the examination, in OHT and POAG patients the response seems to increase with time, with each next flicker period, indicating a slower adjustment of the vessel regulatory mechanisms to the increased tissue perfusion demand. This "conditioning" of the vessel response was not present in controls; the response was constant in all three flicker periods. The similarity of the behaviour pattern between the POAG patients and the OHTs could indicate a role of increased intraocular pressure in the process; both groups had a native IOP significantly higher than the controls. However, in the OHT group the vessel response did reach the level observed in controls in the third flicker period, and also as mentioned above there was no significant difference in mean response between the OHTs and controls. On the other hand, our cohort of POAG patients had significantly lower native IOP level than the OHT group. Being a tertiary referral centre specialized in ocular vascular disorders, it is likely that our cohort of POAG patients there is shifted towards "normal"-tension glaucoma patients, and that mechanisms other than IOP might have played a role. One possibility is that dysregulation of endothelium-derived substances, whose function is altered in normal-tension glaucoma [19, 20], also affects the

neurovascular coupling. Failing to provide adequate perfusion in times of increased need may lead to short-term ischemia and hence to reperfusion damage [12]. The response "conditioning", an increase of response with each next flicker period, might represent an attempt, even though insufficient, of regulatory mechanisms to meet an increased tissue demand.

In the study from 2006 [16], the retinal vessel response dynamics was analyzed in healthy vasospastic and non-vasospastic subjects, and the non-vasospastic group demonstrated a response augmentation in three flicker periods. There are, however, important differences to the present study cohort and design: neither controls nor the patients were subdivided according to their vasospastic propensity, the grouping criteria here was solely the presence of the disease. More importantly, the former study analysed the 1-second vasodilation of the vessels with the goal of capturing the maximal vessel diameter excursion in young healthy subjects. In the present study, an AUC was calculated for three flicker stimulations, thus containing information about the vessel behaviour throughout each entire 20-second flicker period.

An additional weakness of the study is that the RVA device measures the thickness of the blood column and not directly the diameter of the vessels, as they are optically invisible. However, the blood column thickness, and especially its changes, can be viewed as a reliable indicator of the vessel diameter.

The circulation of interest in glaucoma is the choroidal circulation and not the retinal vasculature, apart from the superficial layer of the optic disc tissue. Blood supply from the short-posterior arteries is more relevant to explain the vascular component of glaucoma damage than the temporal retinal arteries, limiting the value of the present findings. It is nevertheless interesting that the neighbouring vascular bed demonstrates such flow alterations, re-affirming the hypothesis that an underlying vascular dysregulation is involved in the pathogenesis of glaucoma.

Systemic medications may have influenced some aspects of ocular blood flow measurements; however, the drug distribution was comparable across groups. The number of patients taking individual drugs, or drug groups, was too small for a meaningful subanalysis. However, as no patient preselection was made based on systemic medication, the composition of the present patient cohorts probably reflects an average composition of patients in the daily practice.

In conclusion, retinal vascular response of untreated POAG patients to repeated flicker light stimulation is both reduced and demonstrates an altered pattern compared to age-matched controls. Future research is required to examine possible diagnostic and therapeutic consequences of this finding.

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Conflict of interest None

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