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Endothelin-1 plasma levels and vascular endothelial dysfunction in primary open angle glaucoma

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ABSTRACT

Aims: To assess the relationship between endothelial dysfunction, endothelin 1 (ET-1) plasma levels and sub-clinical inflammation in primary open angle glaucoma (POAG) patients.
Main methods: We enrolled 40 POAG patients with progressive visual field damage, although well controlled intraocular pressure (IOP) and compared to age and sex matched healthxy subjects. Each patient underwent an ophthalmological examination, a standard achromatic perimetry (SAP), blood sampling to assess ET-1 plasma levels, an objective assessment of cellularity within the anterior chamber (FLARE) and measurement of flow mediated dilation (FMD) with high resolution 2-dimensional ultrasonographic imaging of the brachial artery.
Key findings: At baseline, POAG patients, compared to healthy controls, showed an increase of ET-1 plasma levels: 2.83 ± 0.28 pg/ml vs. 1.75 ± 0.25 pg/ml ($p<0.001$), lower FMD values $4.46 \pm 1.28\%$ vs. $13.18 \pm 2.80\%$ ($p<0.001$) and increased FLARE values 9.98 ± 0.97 photons/ms vs. 5.87 ± 0.64 photons/ms ($p<0.001$). A follow up after 1 year revealed a further increase of ET-1 plasma levels (to 3.68 ± 0.60; $p<0.001$) and decrease of FMD (3.52 ± 1.28; $p>0.001$).
Significance: The increase of ET-1 in POAG patients is related to vascular dysfunction ($r=0.942$; $p=0.001$) and vascular dysfunction is related to sub-clinical intraocular inflammation ($r=0.968$; $p=0.001$). Thus ET-1 and vascular dysfunction related to sub-clinical inflammation may play a key role in determining a progressive visual field damage in POAG patients who present a well-controlled IOP.

Introduction

Glaucoma, a progressive optic neuropathy, is the second largest cause of blindness worldwide (Congdon et al., 2003; Quigley, 2005). Elevated intraocular pressure (IOP) is by far the most widely recognised risk factor that can be treated to slow down the rate of progression of glaucomatous damage (Coleman and Caprioli, 2009). Nevertheless, despite well-controlled IOP, this neurodegenerative disease can still progress (Leske et al., 2007; Mackenzie and Cioffi, 2008). Over the last few years, there has been mounting evidence in literature on the possible role played by the ocular vascular system and associated vascular mediators in glaucoma (Flammer and Mozaffarich, 2007; Grieshaber and Flammer, 2005).

Endothelin-1 (ET-1) has been suggested to be a potential contributor to the pathogenesis of glaucoma (Yorio et al., 2002). ET-1 is the most potent vasoconstrictor known of small and large vessels (Masaki et al., 1991). It is a peptide with 21 amino acid residues, mainly released by the endothelial cells of the arterial, venous and lymphatic vessels (Yanagisawa et al., 1988; Vane et al., 1989). Increased ET-1 plasma levels have been described in normal tension glaucoma (NTG) (Sugiyama et al., 1995; Cellini et al., 1997) but not in studies where primary open angle glaucoma (POAG) patients had stable damage of the visual field (Tezel et al., 1997; Hollo et al., 1998). The fact that aqueous ET-1 levels are increased in POAG (Tezel et al., 1997; Naske et al., 1997) underscores the possible contribution of ET-1 to the pathogenesis of POAG. Furthermore, increased plasma levels of ET-1 were found in POAG patients with progressive disease (Emre et al., 2005).

Given that the endothelium is involved in the control of vascular tone and blood flow (Resch et al., 2009), vascular dysregulation can be a consequence of endothelial dysfunction, so that we can evaluate peripheral vascular endothelial function using a noninvasive and well-established brachial artery ultrasound assessment of endothelium-dependent flow mediated dilation (FMD), an index of vasomotor function (Corretti et al., 2002; Deanfield et al., 2007).

In this study we assessed ET-1 plasma levels, vascular endothelial dysfunction and sub-clinical intraocular inflammation in healthy subjects and POAG patients with progressive visual field deterioration, at baseline and after 1 year of follow up.

Materials and methods

We studied 40 POAG patients (22 males and 18 females; mean age 54.5 ± 10.2 years) in good IOP control, but with progressive visual

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field deterioration, all from the Ophthalmology Service at the S. Orsola-Malpighi Hospital, and 40 healthy controls (20 males and 20 females; mean age 52.9 ± 7.1 years), all recruited amongst visitors to and staff of that service.

The study was conducted in compliance with the Declaration of Helsinki and was approved by the institutional review board and independent ethical committee. The inclusion criteria for POAG patients were: IOP<20 mmHg after treatment with carbonic anhydrase inhibitors eye drops, open anterior chamber angle at gonioscopy, cup/disc ratio ≥ 0.6 and an optic nerve-related visual field loss mean defect (MD) of >6 dB in the last three standard automated perimetry estimations. Criteria for reliability of the visual field tests were ≤ 33% false positive, ≤ 33% false negative and ≤ 20% fixation losses. The definition of visual field defect progression consisted of deepening of an existing scotoma, expansion of an existing scotoma or a new scotoma in a previously normal region of the visual field in the last three tests.

Exclusion criteria were: age > 65 years, smoking, autoimmune disease, consumption of systemic medications such as antihypertensive drugs, cholesterol-lowering agents, aspirin or nitrates, and cardiovascular disease or known cardiovascular risk factors such as hypertension, diabetes mellitus, dyslipidemia, in order to clear the study sample from possible confounders affecting endothelial function. Furthermore, we excluded patients with a history of previous eye surgery. All patients underwent an ophthalmologic examination including visual acuity and IOP assessment, with Goldmann applanation tonometry, corneal thickness evaluation with a Tomey SP3000 pakimeter (Tomey Corp., Nagoya, Japan) biomicroscopy of the anterior and posterior segment and automatic measurement of the C/D area ratio of the optic nerve head with Stratus OCT3 (Zeiss-Humphrey, Dublin, CA).

Standard achromatic perimetry was also performed with a Humphrey Field Analyzer, using a 30-2 full threshold program (Carl Zeiss Meditec, Inc., Dublin, CA, USA). All subjects gave informed written consent before enrolment.

For each subject, before the beginning of the ultrasound measurement of FMD, body mass index, blood sampling to assess lipid profile, fasting serum glucose and ET-1 plasma levels and an objective assessment of cellularity within the anterior chamber (FLARE) were obtained and after a follow up of 1 year, FMD, ET-1 plasma levels and FLARE were repeated.

For ET-1 measurements the plasma samples were drawn from the antecubital vein and collected in a container with EDTA, cooled and stored in ice. Subsequently, the samples were centrifuged at 4 °C and frozen at −25 °C. After centrifugation the extraction was performed using a Sep-column containing C-18 (Peninsula Laboratories, Belmont, CA, USA). ET-1 concentration was determined using a commercial radioimmunoassay (RIA) kit (Peninsula Laboratories, Belmont, CA, USA). For the RIA, samples and standards were incubated with rabbit anti-ET-1 serum for 24 hours at 4 °C. A second 24 hours incubation was made after the addition of an iodinated tracer [^{125} I]-ET-1. Free and bound radioligands were separated with centrifugation and radioactivity in the precipitate was counted with an automatic gamma-counter.

The cellularity of the anterior chamber was evaluated with a laser flare-cell meter (FM 500 Kowa, Tokio, Japan). The flare cell meter consisted of an He-Ne laser beam system, a photomultiplier mounted on a slit-lamp microscope and a computer. The maximum power of the He-Ne laser was 50 μW and the diameter of the focused beam 20 μm as measured in the air. The laser scanned the aqueous humor by means an optical scanner. The sampling window (0.3x0.5 mm) installed in the microscope, was aligned with the laser beam in the aqueous humor. The intensity of light passing through the sampling window was measured by means of a photon- counting photomultiplier and analysed by a computer.

When the laser beam is projected through the sampling window in the anterior chamber it hits the particles (protein and/or cells) in suspension in the aqueous humor and is diffused by these with an intensity proportional to their concentration. It is possible to count and differentiate the number of cells present in the aqueous humor from the proteins as the light diffused by them is greater than that diffused by the fine protein particles (Sawa, 1990).

All subjects underwent measurement of FMD with high resolution 2-dimensional ultrasonographic imaging of the brachial artery by means of a Philips ENVISOR echography machine (Philips Medical Systems, Best, The Netherlands) and a 4–7 MHz linear probe. The FMD technique has been described in detail elsewhere (Corretti et al., 2002).

Best corrected visual acuity (BCVA) was converted into the logarithm of the minimum angle of resolution units (LogMAR) for the statistical analysis and all data were analysed using Student's t test for unpaired data to compare POAG patients and controls and Student's t test for paired data to compare POAG patients at baseline and after 1 year. In those cases in which the standard deviation of the two groups under examination appeared to be significantly different, t test with Welch correction was used. Spearman's correlation test was employed to evaluate correlations between ET-1, FMD, FLARE and MD visual field index.

All statistical analyses were performed using GraphPad InStat version 3.05 for Windows (GraphPad Software, San Diego, California, USA). A $p<0.05$ value was considered to be statistically significant.

Results

Table 1 summarizes the demographic and clinical characteristics of the POAG patients and healthy subjects. Only the C/D ratio parameter is significantly different between the two groups ($p<0.001$).

At baseline in POAG patients, compared to healthy controls, we found an increase of ET-1 plasma levels (2.83 ± 0.28 vs. 1.75 ± 0.25 pg/ml; $p<0.001$), lower FMD values (4.46 ± 1.28 vs. 13.18 ± 2.80%; $p<0.001$), higher FLARE values (9.98 ± 0.97 vs. 5.87 ± 0.64 photons/ms ; $p<0.001$) and higher MD values: (9.61 ± 3.47 vs. 2.66 ± 1.39 dB; $p>0.001$) (Table 2).

After 1 year follow-up, among POAG patients, we observed a new increase of ET-1 plasma levels (3.68 ± 0.60 vs. 2.83 ± 0.28 pg/ml; $p<0.001$), a further decrease of FMD values (3.52 ± 1.28 vs. 4.46 ± 1.28%: $p<0.039$), unchanged FLARE values (9.93 ± 0.85 vs. 9.98 ± 0.97 photons/ms; $p<0.736$) and higher MD values (12.15 ± 3.49 vs. 9.61 ± 3.47 dB; $p<0.007$) (Table 2).

The IOP in POAG group at baseline was not significantly different from that measured in the control group (17.42 ± 1.30 mmHg vs. 17.44 ± 1.76 mmHg; $p<0.477$) or from that assessed in the same group 1 year later (17.47 ± 0.86 mmHg vs. 17.42 ± 1.30 mmHg; $p<0.433$) (Table 2).

Table 1
Demographic and clinical characteristics in the study groups at baseline.

	POAG	Controls	P value
Number of patients (M:F)	40 (22:18)	40 (20:20)	0.750
Age (years)	54.5 ± 10.2	52.9 ± 7.1	0.834
Body mass index (Kg/m²)	25.77 ± 2.73	23.85 ± 3.47	0.103
Systolic blood pressure (mmHg)	125.13 ± 15.03	123.00 ± 15.22	0.702
Diastolic blood pressure (mmHg)	76.80 ± 7.45	78.67 ± 7.66	0.504
Total cholesterol (mg/dl)	194.42 ± 29.21	188.73 ± 24.41	0.567
HDL-cholesterol (mg/dl)	53.37 ± 13.16	48.66 ± 12.38	0.321
LDL-cholesterol (mg/dl)	116.22 ± 26.81	115.70 ± 24.85	0.956
Triglycerides (mg/dl)	107.85 ± 62.77	103.54 ± 47.75	0.834
Serum glucose (mg/dl)	92.30 ± 7.97	93.29 ± 9.20	0.754
BCVA (LogMAR)	0.89 ± 0.2	1.0 ± 0.1	0.257
C/D ratio	0.69 ± 0.1	0.29 ± 0.13	0.001
Corneal thickness (μm)	549.6 ± 15.25	551.6 ± 12.57	0.230

All data represent mean values ± Sd.
POAG = primary open angle glaucoma; M = male; F = female; HDL = high density lipoprotein; LDL = low density lipoprotein; ET-1 = endothelin-1; BCVA = best corrected visual acuity; IOP = intraocular pressure; C/D = cup and disc ratio.

Table 2
Difference in vascular dysfunction, visual field index, IOP and aqueos flare data between POAG patients and healthy controls at baseline and among POAG patients after 1 year.

	Controls (baseline)	POAG (baseline)	P value	POAG (follow-up)	P value
ET-1 (pg/ml)	1.75 ± 0.25	2.83 ± 0.28	0.001	3.68 ± 0.60	0.001
FMD %	13.18 ± 2.80	4.46 ± 1.28	0.001	3.52 ± 1.28	0.039
FLARE (photons/ms)	5.87 ± 0.64	9.98 ± 0.97	0.001	9.93 ± 0.85	0.736
MD (dB)	2.66 ± 1.39	9.61 ± 3.47	0.001	12.15 ± 3.49	0.007
IOP (mm/Hg)	17.44 ± 1.76	17.42 ± 1.30	0.477	17.47 ± 0.86	0.433

The number of patients is 40.
ET-1 = endothelin-1; FMD = flow mediated dilation; MD = visual field index "mean defect"; IOP = intraocular pressure; FLARE = aqueous flare.

Spearman's rank correlation test (Table 3) showed that the increase of ET-1 in POAG is related to vascular dysfunction ($r = 0.942$; $p = 0.001$) and intraocular inflammation ($r = 0.968$; $p = 0.001$). Vascular dysfunction is related to intraocular inflammation ($r = 0.953$; $p = 0.001$) and to ET-1 plasma levels ($r = 0.942$; $p = 0.001$). Furthermore the progression of the visual field damage is related to ET-1 plasma levels ($r = 0.746$; $p = 0.002$) and to vascular dysfunction ($r = 0.690$; $p = 0.001$) but it is not related neither to IOP ($r = -0.199$; $p = 0.401$) nor to intraocular inflammation ($r = -0129$; $p = 0.527$). Finally the values of ET-1 ($r = -0.046$; $p = 0.845$), FMD ($r = -344$; $p = 0.137$) and FLARE ($r = 0.072$; $p = 0.762$) are not age-related.

Discussion

Glaucoma is a group of diseases featuring multiple risk factors that may act with variable importance and severity. The IOP is by far the most widely recognized risk factor and usually the only one that is therapeutically targeted in the clinical setting (Weinreb and Khaw, 2004; Levin, 2005); nevertheless increasing interest has been focused on a better understanding of the ocular hemodynamic in glaucoma patients.

Our study highlighted that POAG patients showed increased ET-1 plasma levels ($p<0.001$), a systemic vascular endothelial dysfunction detectable with a lowered FMD ($p<0.001$) and an increase of aqueous flare ($p<0.001$), compared to control group. One year after the original baseline measurements, plasma ET-1 levels and MD measurements progressed and FMD values decreased further in POAG patients, despite the fact that IOP was controlled and FLARE did not show any additional increase.

Furthermore we demonstrated that the increase of ET-1 in our patients was related to vascular dysfunction ($r = 0.942$; $p = 0.001$), that vascular dysfunction was related to sub-clinical intraocular inflammation ($r = 0.953$; $p = 0.001$), and that increase in ET-1 levels was related to the progression of both the visual field damage ($r = 0.746$; $p = 0.002$) and vascular dysfunction ($r = 0.690$; $p = 0.001$), as revealed by Spearman's rank correlation test.

Table 3
Correlation between FMD, ET-1, FLARE, MD and IOP in POAG patients.

Spearman's rank correlation test	FMD	FLARE	IOP	MD
ET-1	r = 0.942	r = 0.968	r = 0.118	r = 0.746
	p = 0.001	p = 0.001	p = 0.619	p = 0.002
FMD		r = 0.953	r = 0.044	r = 0.690
		p = 0.001	p = 0.852	p = 0.001
FLARE			r = 0.082	r = -0.129
			p = 0.728	p = 0.527
IOP				r = -0.199
				p = 0.401

The number of patients is 40.
ET-1 = endothelin-1; FMD = flow mediated dilation; MD = visual field index "mean defect"; IOP = intraocular pressure; FLARE = aqueous flare.

In past literature the importance of the vascular factor in the onset and progression of glaucomatous optic neuropathy was emphasized not only for NTG patients (Sugiyama et al., 1995; Cellini et al., 1997; Butt et al., 1997), but also for POAG patients (Flammer, 1994; Hayreh, 1997) and many recent studies have confirmed the validity of that hypothesis (Emre et al., 2005; Flammer et al., 2002; Pache et al., 2002; Galassi et al., 2003; Zink et al., 2003; Fuchsjäger-Mayrl et al., 2004).

The main mediators acting on ocular blood circulation are nitric oxide (NO), synthesized by the endothelial isoform of nitric oxide synthase enzyme and ET-1 that, interacting with ETA receptors on vascular smooth muscle cells, causes a marked vasoconstriction (Yanagisawa et al., 1988; Arai et al., 1990).

At the ocular level, ET-1 can reduce the ocular blood flow in the retina, choroid and optic nerve head (Schmetterer et al., 1997; Polak et al., 2001, 2003), causing a vasoconstriction in the posterior ciliary arteries, but also, may exert direct receptor mediated effects on retinal ganglion cells, where both ETA and ETB receptors are expressed, so it is possible that increased ET-1 levels in glaucoma play a role in the astrocyte proliferation that occurs in glaucomatous optic neuropathy (Zimmermann, 1997). Finally, there is evidence that the increased aqueous levels of ET-1 in POAG reduce aqueous humor outflow by inducing contraction of trabecular meshwork fibers (Cellini et al., 2006).

Endothelium is a complex organ that regulates hemostasis, inflammation, and vascular tone, which is highly dependent on both the balance between NO and ET-1 (Moncada and Higgs, 1993; Webb and Haynes, 1993) and the preserved function of the vascular endothelial cells (Springer, 1995).

Moreover chronic endothelial dysfunction is characterized by an altered turnover of the endothelial cells due to a reduced availability of endothelial progenitor cells (Asahara et al., 1997; Hill et al., 2003; Fadini et al., 2009), with a subsequent reduced biosynthesis and/or bioavailability of NO and raised levels of superoxide anions and ET-1.

FMD is a standard, non invasive, repeatable and endothelium-dependent technique that is used for an in vivo assessment of peripheral vascular endothelial functionality. It is often used to determine cardiovascular disease risk factors in cardiovascular research (Anderson et al., 2000), but more recently has also been adopted in ophthalmological studies to investigate peripheral vascular endothelial functionality in glaucoma patients (Su et al., 2008), in NTG (Su et al., 2006) and pseudoexfoliative syndrome (Atalar et al, 2006).

The presence of normal range ET-1 plasma levels in POAG patients with stable visual field (Cellini et al., 1997), together with altered ocular hemodynamic on color Doppler imaging examination (Cellini et al., 1996) suggests that vascular endothelial dysfunction is an event that comes before the increase of plasmatic ET-1 levels.

Furthermore experimental studies on systemic hypertension show that production of vasoconstrictor substances by cyclooxygenase, including vasoconstrictor prostanoids and reactive oxygen species, contributes to the pathogenesis of endothelial dysfunction in this disease (Luscher and Vanhoutte, 1986; Yang et al., 2003).

So endothelial dysfunction, which might be at least partially caused by a sub- clinical inflammatory status may play a key role in determining a progressive visual field damage in POAG patients who present a well controlled IOP.

Conclusion

Our study highlights that POAG patients with progressive visual field deterioration, despite a good IOP control, show increased ET-1 plasma levels with a vascular dysfunction and increased FLARE values.

So in the causative mechanism of progression of visual field damage in POAG patients, who present a well controlled IOP, we speculate that a sub-clinical inflammatory status may act as a trigger for the endothelial dysfunction and for the alteration of ET-1 plasma levels.

Although these findings are suggestive, further studies are needed to identify the exact causes of endothelial dysfunction and to find the most suitable therapeutic strategies.

Conflict of interest statement

The authors declare there are no conflicts of interest.

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