Increased plasma endothelin-1 levels in patients with progressive open angle glaucoma

M Emre, S Orgül, T Haufschild, S G Shaw, J Flammer

Aim: To compare the plasma levels of endothelin-1 (ET-1) between patients with primary open angle glaucoma with visual field progression despite normal or normalised intraocular pressure and patients with stable visual fields in a retrospective study.

Methods: The progressive group consisted of 16 primary open angle glaucoma patients and the group with stable visual field consisted of 15 patients. After a 30 minute rest in a supine position, venous blood was obtained for ET-1 dosing. Difference in the plasma level of ET-1 between two groups was compared by means of analysis of covariance (ANCOVA), including age, sex, and mean arterial blood pressure as covariates.

Results: ET-1 plasma levels were found to be significantly increased in patients with deteriorating (3.47 (SD 0.75) pg/ml) glaucoma when compared to those with stable (2.59 (SD 0.54) pg/ml) visual fields (p = 0.0007).

Conclusions: Glaucoma patients with visual field progression in spite of normal or normalised intraocular pressure have been found to have increased plasma endothelin-1 levels. It remains to be determined if this is a secondary phenomenon or whether it may have a role in the progression of glaucomatous damage.

E ndothelin-1 (ET-1) has been suggested to be a potential contributor to the pathogenesis of glaucoma. ET-1 is one of the most potent vasoconstrictors and was first described by Yanagisawa and colleagues in 1988. Although ET-1 appears to act mainly as a local paracrine/autocrine peptide, circulating levels of endothelin seem also to have a biological significance, especially in pathological states of increased serum concentration.

Increased plasma ET-1 levels have been described in normal tension glaucoma patients, although this was not confirmed in all studies with normal tension glaucoma patients or in studies with high tension glaucoma patients. The fact that aqueous ET-1 concentration is increased in primary open angle glaucoma and in animal models of glaucoma underscores the possible contribution of endothelin to the pathogenesis of primary open angle glaucoma. Furthermore, chronic administration of ET-1 has been shown to produce an optic neuropathy similar to glaucoma.

Because an active role of ET-1 in POAG would suppose a higher level in patients with active disease, we compared the plasma levels of ET-1 in POAG patients with progressive visual field deterioration to POAG patients with a stable damage.

PATIENTS AND METHODS

This study adhered to the tenets of the Declaration of Helsinki and was approved by the local ethics committee. Informed consent for the use of their clinical data in a scientific publication was obtained from each patient. Thirty one primary open angle glaucoma patients (seven men and 24 women), including normal tension glaucoma patients, were selected. Patients with closed iridocorneal angles, evidence of secondary glaucoma, pseudoexfoliation, pigmentary dispersion, a history of intraocular surgery (except for filtration surgery), any form of retinal or neuroophthalmologic disorder, a history of chronic systemic medication or disease, especially diabetes mellitus, systemic hypertension, occlusive vascular disorders, chronic heart failure, renal failure, glomerulonephritis, and autoimmune diseases, were not included.

Eyes had to have a visual acuity of 20/30 or better and no clinical evidence of opacity of the media (nuclear sclerotic cataract, or the development of any degree of posterior subcapsular cataract) at the time the patient was included into the study. No attempt was done to wash out antiglaucoma medication. However, in order to control for the influence of medical or surgical glaucoma therapy, only patients using the same topical antiglaucoma medication in both eyes and throughout the entire follow up period for which stability or progression in visual field damage was evaluated, or patients subjected to filtration surgery prior to this observation period were included.

In each patient a diurnal intraocular pressure (IOP) curve (before arising from bed at 6.00 am, 8.00 am, 11.00 am, 4.00 pm, and 10.00 pm) was obtained in both eyes the day ET-1 was given. Only patients showing no readings above 21 mm Hg were considered for the present analysis.

All patients had typical glaucomatous disc and visual field damage. Only data from patients experienced in visual field testing were considered. After excluding the first fields from the series available, retrospective information for at least five consecutive visual field examinations had to be available in each patient for evaluation of progression. No alteration in treatment during this period was tolerated. The patients had 3 mm or larger pupil diameters when their fields were plotted and fields with poor reliability (fixation loss exceeding 20% and false-positive or false-negative errors exceeding 33%) were not considered.

Visual field examinations had been performed with the program G1 on the Octopus Visual Field Analyzer (Interzeag, Schlieren, Switzerland). The criteria for glaucomatous visual field defects were a cluster of three points (except rim points) in at least one hemifield reduced by 5 dB or greater, and including at least one point reduced by 10 dB or greater; a cluster of two points reduced by 10 dB or

**Abbreviations:** ET-1, endothelin-1; DBP, diastolic blood pressure; IOP, intraocular pressure; MBP, mean blood pressure; SBP, systolic blood pressure
greater; or three adjacent points on the nasal horizontal meridian that differed by 5 dB or greater from their mirror points on the opposite side of the meridian.

The definition of visual field progression consisted of deepening of an existing scotoma, expansion of an existing scotoma, or a fresh scotoma in a previously normal part of the visual field, in the three last fields of the selected series per eye. A deepening or expansion of an existing scotoma was diagnosed if two adjacent points had declined 10 dB from their original values, and a new scotoma was diagnosed if an alteration meeting the criteria for a visual field defect occurred in a previously normal part of the field. In patients with only one eye showing progressive damage, this eye was selected for further evaluation; when both eyes showed progression, or no progression had occurred in both eyes, one randomly chosen eye was considered for further evaluation.

ET-1 plasma levels were determined by a specific radioimmunoassay, as described by d’Uscio et al. Venous EDTA blood samples (20 ml) were taken after 30 minutes of rest in a supine position at room temperature at about 8.00 am. The blood samples were stored immediately on ice and cooled centrifugation at 4°C was performed for 10 minutes. Plasma was separated at 4°C and kept at −80°C until assay. Extraction was performed by adsorption on 500 mg SepPak Vac C18 cartridges (Millipore Ltd, Watford, UK). Columns were preactivated by successive washes with 5 ml of 86% ethanol in 4% acetic acid, 5 ml of methanol, 5 ml of sterile distilled water, and 5 ml of 4% acetic acid. A 2 ml plasma sample acidified with 6 ml of 4% acetic acid was then applied on the column with the flow rate of 3 ml/min. The columns were then washed with 18 ml of sterile distilled water, 1.8 ml ethyl acetate, and 18 ml of 24% ethanol in 4% acetic acid before ET was eluted with 86% ethanol in 4% acetic acid. The eluate was dried under nitrogen at 37°C and redissolved in before ET was eluted with 86% ethanol in 4% acetic acid. The columns distilled water, and 5 ml of 4% acetic acid. A 2 ml plasma were preactivated by successive washes with 5 ml of 86% sample were acidified according to the manufacturer’s instructions and then further diluted ethanol in 4% acetic acid, 5 ml of methanol, 5 ml of sterile in 230 μl of assay buffer composed of 0.1% phosphate buffer distilled water, and 5 ml of 4% acetic acid. A 2 ml plasma (pH 7.4), 0.05 mol/l NaCl, 0.1% Triton X-100, 0.02% sodium sample acidified with 6 ml of 4% acetic acid was then applied on the azide, and 0.1% BSA. The radioimmunoassay of plasma ET column with the flow rate of 3 ml/min. The columns was performed using synthetic human/porcine ET-1 (Sigma were then washed with 18 ml of sterile distilled water, 1.8 ml Chemical Co, St Louis, MO, USA), a rabbit antibody against ethyl acetate, and 18 ml of 24% ethanol in 4% acetic acid synthetic ET (Peninsula Laboratories, San Carlos, CA, USA), and before ET was eluted with 86% ethanol in 4% acetic acid 125I-ET-1 (Amersham, Freiburg, Germany). The antibody before ET was eluted with 86% ethanol in 4% acetic acid has 100% cross reactivity with ET-1, 7% with ET-2 and ET-3, before ET was eluted with 86% ethanol in 4% acetic acid 17% with big endothelin-1, and no cross reactivity with other before ET was eluted with 86% ethanol in 4% acetic acid peptides. The anti-ET antibody was reconstituted according to the standardised instructions and then further diluted to the manufacturer’s instructions and then further diluted 1:3.5 with the assay buffer before adding 100 μl to the assay buffer before adding 100 μl to the standards or the reconstituted plasma samples (100 μl) the standards or the reconstituted plasma samples (100 μl) analysed in duplicate. After 24 hours of incubation, 100 μl analysed in duplicate. After 24 hours of incubation, 100 μl of of 125I-ET-1 (10 to 12×105 cpm per tube) was added, and incubation was allowed to continue for an additional 24 hours. The separation of bound and free antigen was incubation was allowed to continue for an additional 24 hours. performed with a second antibody method, and pellets were The antibody was 100% cross reactive with ET-1, 7% with ET-2 and ET-3, counted by a gamma counter (Canberra Packard). Reference velocities with other peptides. The anti-ET antibody was reconstituted according to the values were calculated as the mean (standard deviation) in our laboratory are 1.42 reference value established in our laboratory, and MBP as covariates into the model (ANOVA: F (1,25) = 13.94281; p = 0.0008). This difference was still significant after controlling for the interaction of sex and including age after controlling for the interaction of sex and including age and MBP as covariates into the model (ANOVA: F (1,25) = 14.95; p = 0.0007). Sex had a borderline significant effect (ANOVA: F (1,25) = 4.25; p = 0.0497), but the interaction effect (ANOVA: F (1,25) = 4.25; p = 0.0497), but the interaction between sex and disease progression was not significant (ANOVA: F (1,25) = 1.84; p = 0.19).

**DISCUSSION**

The present study suggests an increased plasma level of ET-1 in primary open angle glaucoma patients with progressive damage when compared with primary open angle glaucoma patients with stable visual fields. This difference was independent of sex, age, and mean blood pressure. Furthermore, the values obtained among patients with a stable visual field were above the reference values established in our laboratory. A possible explanation for these findings could be that ET-1 may contribute primarily to the damaging process in glaucoma, but also remain increased secondarily to the

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Progressive group</th>
<th>Stable group</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>121.9 (18.9)</td>
<td>114.8 (12.3)</td>
<td>0.23</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>75.6 (14.0)</td>
<td>71.9 (8.0)</td>
<td>0.38</td>
</tr>
<tr>
<td>MBP (mm Hg)</td>
<td>91.0 (15.0)</td>
<td>86.2 (6.4)</td>
<td>0.26</td>
</tr>
<tr>
<td>Follow up (months)</td>
<td>48.4 (16.8)</td>
<td>50.2 (21.6)</td>
<td>0.80</td>
</tr>
<tr>
<td>IOP at baseline (mm Hg)</td>
<td>17.0 (4.3)</td>
<td>16.7 (4.8)</td>
<td>0.84</td>
</tr>
<tr>
<td>MD at baseline (dB)</td>
<td>6.4 (4.6)</td>
<td>5.0 (2.6)</td>
<td>0.30</td>
</tr>
<tr>
<td>MD at the end of follow up (dB)</td>
<td>12.0 (5.2)</td>
<td>4.8 (3.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Number of visual fields</td>
<td>6.6 (1.6)</td>
<td>7.4 (2.9)</td>
<td>0.36</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The present study suggests an increased plasma level of ET-1 in primary open angle glaucoma patients with progressive damage when compared with primary open angle glaucoma patients with stable visual fields. This difference was independent of sex, age, and mean blood pressure. Furthermore, the values obtained among patients with a stable visual field were above the reference values established in our laboratory. A possible explanation for these findings could be that ET-1 may contribute primarily to the damaging process in glaucoma, but also remain increased secondarily to the
damage. An alternative explanation would be that some patients in the group with stable visual fields were in an active stage of their disease, but did not yet satisfy the criteria used to define progression in visual field damage.

The cause for the increased levels of circulating ET-1 in primary open angle glaucoma patients is not clear. One may be tempted to explain the link between systemic levels of endothelin and glaucoma with the association of glaucoma and the primary vasospastic syndrome. 2 High plasma levels of ET-1 have been described in diseases associated with vascular dysregulation such as cerebral vasospasm after subarachnoid haemorrhages, 14, 15 Raynaud’s phenomenon, 16, 17 and ischaemic heart disease. 18, 19 Furthermore, it is believed that in some patients such as in individuals with drug induced coronary spasms, an abnormal response of the vascular endothelium to certain stimuli can lead to abnormally high levels of ET-1 and consequent vasoconstriction. 20 An abnormal endothelial function with an abnormal release of ET-1 after a vasospastic stimulus has been suggested to be related to the genesis of vasospasm in glaucoma. 21 Kaiser et al. also demonstrated a faulty regulatory mechanism in the production of ET-1 in normal tension glaucoma: the physiologic increase in plasma ET-1 levels observed when subjects moved from a supine to an upright position was absent in the patients with normal tension glaucoma. 22 Finally, the vascular response to ET-1 has been shown to be enhanced in subcutaneous resistance arteries from patients with NTG, 23 and glaucoma patients with lower blood pressure values react more sensitively to ET-1. 24 As a result of vascular events, ET may in turn have further vascular effects, including ET\textsubscript{A} mediated rapid vasoconstriction and ET\textsubscript{B} induced vasodilation mediated by nitric oxide and possibly TNF-\textalpha. 25, 26 but increased ET-1 levels may also exert direct receptor mediated effects on retinal ganglion cells and resident glial cells. Retinal ganglion cell loss in glaucomatous optic neuropathy is associated with a disruption in anterograde axonal transport. 27, 28 Theoretically, this can result either from nerve compression 29 or from ischaemia. 30 However, the effect of direct compression or ischaemia on axonal transport has been suggested to involve the loss of linear microtubule arrays in exposed axons, 31, 32 whereas in glaucoma a rather selective dysregulation of axonal transport seems to occur. 33 It has been hypothesised that mechanisms contributing to the perturbation of anterograde axonal transport could involve ET-1. 34, 35 Indeed, intravitreal ET\textsubscript{A} induces a significant impairment of anterograde axonal transport in retinal ganglion cells, 36 but, interestingly, ET\textsubscript{A} receptor mediated vasoconstriction seems not be a prerequisite for the effect of ET on axonal transport, and evidence has been shown that the observed perturbation might result from the stimulation of ET\textsubscript{B} receptors. Both receptor types ET\textsubscript{A} and ET\textsubscript{B} are expressed in retinal ganglion cells. 37

### Table 2 Topical glaucoma treatment

<table>
<thead>
<tr>
<th>Drug</th>
<th>Progressive group</th>
<th>Stabile group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timolol</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Betaxolol</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Dorzolamide</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Latanoprost</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Brinzolamide</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Dorzolamide + Timolol</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Dorzolamide + Latanoprost</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Dorzolamide + Brimonidine</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Timolol + Latanoprost</td>
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<td>1</td>
</tr>
<tr>
<td>Timolol + Pilocarpine</td>
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<td>0</td>
</tr>
<tr>
<td>Betaxolol + Brinzolamide</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

**Figure 1** Plasma concentration of ET-1 in patients with glaucoma and without visual field progression (triangle = mean; box = standard error of the mean; whiskers = standard deviation).

Alternatively, circulating levels of ET-1 may be linked indirectly to the loss of retinal ganglion cells in glaucoma. It has been shown that, through activation of both ET\textsubscript{A} and ET\textsubscript{B} receptors, ET-1 can induce astroglial proliferation in cultured astrocytes. 38 It is, therefore, possible that increased ET-1 levels in glaucoma play a role in the astrogial proliferation that occurs in glaucomatous optic neuropathy. 39, 40 Indeed, astroglia occurs not only in human glaucomatous optic nerve neuropathy, 41 but also in animals with experimentally increased intraocular pressure. 42, 43 Hypothetically, normal interaction between glia and neurons may be disturbed during astrocytosis, which may enhance the rate of neuronal loss during glaucomatous damage.

The present study cannot conclusively prove the direct implication of ET-1 in the pathogenic process of glaucomatous optic neuropathy. This would require a prospective study with repeated dosing of plasma levels of ET-1 and, ideally, the assessment of the effect of endothelin receptor blockers.

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### REFERENCES


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