Nitric oxide proxies and ocular perfusion pressure in primary open angle glaucoma

F Galassi, G Renieri, A Sodi, F Uccì, L Vannozzi, E Masini

Background: To investigate the levels of nitric oxide (NO) markers in plasma and aqueous humour of patients with primary open angle glaucoma (POAG) and their relation to ocular perfusion pressure.

Methods: Cyclic guanosine monophosphate (cGMP) and nitrite (NO$_2^-$) were determined in plasma and aqueous humour of 38 patients with POAG and 46 controls. Blood pressure and IOP were measured to calculate ocular perfusion pressure (PP).

Results: cGMP and NO$_2^-$ plasma levels were significantly decreased in glaucoma patients compared with controls (p = 0.001 v p = 0.004). In the aqueous humour of subjects with POAG, cGMP and NO$_2^-$ concentrations were also lower than in normal eyes (v = 0.0001 v = 0.001). There was a linear association between cGMP in plasma and aqueous humour in glaucomas and controls (r = 0.514, p = 0.029 and r = 0.558, p = 0.004) and this relation differed in the two groups (p = 0.003). Considering glaucoma patients with controls, a positive correlation was found between cGMP and PP (r = 0.379, p = 0.01) and between NO$_2^-$ and PP (r = 0.339, p = 0.040). The cGMP/PP correlation was of borderline statistical significance in controls (p = 0.050), whereas it did not attain statistical significance in POAG, as well as the association between NO$_2^-$ and PP when glaucomas and controls were considered separately.

Conclusions: The authors found alterations of NO markers in the plasma and aqueous humour of glaucoma patients. Primary or secondary impaired NO balance could alter ocular perfusion pressure.

Nitric oxide (NO) is synthesised in endothelial cells by type III isoform of nitric oxide synthase (NOS) and, under basal conditions of flow, it sustains a constant vasodilator tone in the vascular system. Changes in the environment, such as increased stress or hypoxia, can stimulate NO synthesis and the local regulation of vascular tone and blood flow.

Abundant NOS immunoreactivity has been found in the optic nerve head (ONH) vasculature. At this level an increase of NOS in the vascular endothelium has been hypothesised to be neuroprotective by promoting vasodilation and tissue blood flow. Intravenous infusion of NOS-inhibitor l-arginine (L-NMMA), a NOS inhibitor, provoked a reduction of ONH blood flow in healthy subjects. Another work reported that the NO donor 5-isosorbide monoamine significantly increases ONH blood flow in normal subjects mainly causing an increment in blood volume, and an elective sensitivity of the ONH circulation to nitrates has been postulated.

Tissue ischaemia has been involved in the pathogenesis of glaucomatous optic neuropathy and an altered circulation has been indicated in some glaucoma patients. Plasma and aqueous humour levels of cGMP, an indirect indicator of NO, were decreased in patients with normal tension glaucoma. The same glaucomatous group showed lower systolic and diastolic velocities of the ophthalmic artery when examined with colour Doppler imaging. In POAG, abnormal IOP and vascular dysregulation might together determine optic nerve damage, both reducing ocular perfusion.

As NO can modulate IOP and ocular circulation, and contrasting data have been published recently on NO production in glaucoma patients, we wanted to elucidate whether NO formation is disturbed in hypertensive POAG and whether it influences ocular perfusion pressure. We therefore investigated the levels of NO$_2^-$, the stable end product of NO metabolism, and of cGMP, the intracellular mediator of NO action, in aqueous humour and plasma of patients with POAG and controls.

**MATERIALS AND METHODS**

**Study population**

Thirty eight POAG patients were enrolled in the study together with 46 control subjects referred to our clinic for cataract surgery. Patients and controls were matched for age, sex, and blood pressure (table 1). The following exclusion criteria were used: any ocular disease except senile cataract or POAG; diabetes; infections; cardiovascular pathologies including hypertension and atherosclerotic lesions determining haemodynamically significant stenosis; assumption of non-steroidal anti-inflammatory drugs; steroids; ACE inhibitors, or NO donors. All subjects had to follow a nitrate free diet for three days before blood extraction.

Glaucoma patients had IOP higher than 21 mm Hg without antiglaucomatous drug, open anterior chamber angle, glaucomatous disc damage (cup:disc ratio ≥0.6), and glaucomatous field loss on the Humphrey perimeter, 30-2 full threshold programme (Humphrey Instruments, San Leandro, CA, USA). Visual field defects were included in the stage I–III, according to Aulhorn’s classification modified by Greve. All control subjects had a normal ocular examination.

Written informed consent was obtained from each subject and the tenets of the Declaration of Helsinki were observed.

**Measurement of cGMP and NO$_2^-$ in the plasma and in the aqueous humour**

Blood samples of 3 ml were collected from a forearm vein of every patient and immediately subjected to plasma separation and stored at −20°C.

**Abbreviations:** cGMP, cyclic guanosine monophosphate; NO, nitric oxide; NOS, nitric oxide synthase; POAG, primary open angle glaucoma; PP, perfusion pressure.
Glaucoma patients and controls admitted to our clinic for cataract surgery, who met the aforementioned inclusion criteria, were also recruited for cGMP and $\text{NO}_2^-$ determination in the aqueous humour. Preoperatively, the same antibiotic and the same standard mydriatic protocol was used (three or four drops of an association of tropicamide 1% and phenylephrine 2.5%) for glaucomas and controls, so the two subgroups differed only for the antiglaucomatous treatment (timolol 0.50% twice a day). Cataract extraction was performed with a phacoemulsification technique. Before performing the clear corneal tunnel, 200–400 µl of aqueous humour was quickly withdrawn by means of a paracentesis, and of a viscoelastic substance. The paracentesis were always performed by the same surgeon with a tuberculine needle on a 1 ml syringe. Ten µl of a solution of $10^{-5}$ M of isobutyl methylxantine (IBMX), a phosphodiesterase inhibitor, was added to each sample. All samples were stored at $-20^\circ\text{C}$.

NO levels were evaluated in plasma and aqueous humour by measuring the concentration of $\text{NO}_2^-$ and of cGMP.

$\text{NO}_2^-$ was measured spectrophotometrically by the Griess reaction, values were obtained by comparison with standard concentrations of sodium nitrite and expressed as micromoles of $\text{NO}_2^-$ per milligram of protein.

cGMP levels were measured in plasma and aqueous humour after the extraction with 10% trichloroacetic acid and of cGMP.

The association between plasmatic and aqueous humour cGMP concentration was also statistically significant when calculated in the two groups separately (POAG group: $n = 18$, $r = 0.514$, $p = 0.029$; control group: $n = 25$, $r = 0.358$, $p = 0.004$) (fig 5).

The relation between plasma and aqueous cGMP significantly differed in glaucoma and control patients (overall test of coincidence, $p = 0.003$; comparison of slopes, $p = 0.001$).

### Table 1  Demographic characteristics of patients enrolled in the study

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>POAG</th>
<th>$p$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>46</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Mean (SD) age</td>
<td>71 (8.31)</td>
<td>69.4 (8.43)</td>
<td>0.394</td>
</tr>
<tr>
<td>Sex</td>
<td>25 women</td>
<td>20 women</td>
<td>0.950</td>
</tr>
<tr>
<td>21 men</td>
<td>18 men</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD) blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>139.6 (14.24)</td>
<td>139.1 (11.71)</td>
<td>0.863</td>
</tr>
<tr>
<td>Diastolic</td>
<td>79.5 (6.78)</td>
<td>78.3 (11.71)</td>
<td>0.559</td>
</tr>
<tr>
<td>IOP (mm Hg)</td>
<td>14.1 (2.8)</td>
<td>16.6 (5.1)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

In control subjects and in patients with bilateral glaucoma the eye considered for PP calculation was chosen by random.

**Statistical analysis**

Results are shown as mean (standard deviation, SD). Statistical comparisons were conducted using the Student’s $t$ test for unpaired observations. Linear regression and correlation were used to analyse the association between cGMP plasma and aqueous humour levels and between cGMP, $\text{NO}_2^-$ plasma concentration and PP. Comparisons between regression lines of glaucomas and controls were performed with the overall test for coincidence. A $p$ value of $\leq 0.05$ was considered significant.

### RESULTS  

**cGMP and $\text{NO}_2^-$ plasma levels**

Forty six controls and 38 POAG subjects were eligible for cGMP assessment and among them, respectively, 19 and 23 also underwent $\text{NO}_2^-$ determination. cGMP plasma concentration was significantly lower in glaucoma patients than in normals ($p = 0.001$, fig 1).

Also $\text{NO}_2^-$ plasma levels were decreased in POAG patients compared with controls ($p = 0.004$, fig 2).

**cGMP and $\text{NO}_2^-$ levels in the aqueous humour**

Aqueous humour samples were collected from 18 POAG patients and 25 subjects with cataract only. The glaucoma patients had a good tonometric control with therapy. Preoperative IOP statistically differed in the two groups (16 (4.33) v 13 (3.15) mm Hg; $p = 0.012$).

In glaucomatous eyes, cGMP mean concentration was significantly lower than in control eyes ($p = 0.0001$, fig 3).

$\text{NO}_2^-$ amount was determined in the aqueous humour every time we could safely withdraw a sufficient volume from the anterior chamber of the individual eye. $\text{NO}_2^-$ was also notably reduced in eyes in POAG when compared with normotensive eyes ($p = 0.001$, fig 4).

### Association between cGMP in plasma and aqueous humour

A linear correlation between cGMP levels in plasma and aqueous humour ($n = 43$, $r = 0.565$, $p < 0.001$) was found. The association between plasmatic and aqueous humour cGMP concentrations was also statistically significant when calculated in the two groups separately (POAG group: $n = 18$, $r = 0.514$, $p = 0.029$; control group: $n = 25$, $r = 0.358$, $p = 0.004$) (fig 5).

The relation between plasma and aqueous cGMP significantly differed in glaucoma and control patients (overall test of coincidence, $p = 0.003$; comparison of slopes, $p = 0.001$).

**Figure 1**  

cGMP plasma levels in control (20.59 68 (697 86) pmoles/mg protein) and glaucoma subjects (1568 62 (625 55) pmoles/mg protein). Values are mean (SD); $p = 0.001$ by the Student’s $t$ test for unpaired data.

---

**Ocular perfusion pressure**

Immediately before collecting the blood sample, the blood pressure was measured by sphygmomanometry. Although patients with a previous medical diagnosis of arterial hypertension were not enrolled at all in the study, we also excluded for ocular perfusion pressure evaluation subjects who had arterial pressure higher than 145/90 mm Hg before plasma withdrawal.

Intraocular pressure was assessed by Goldmann applanation tonometry (Haag Streit, Bern, Switzerland). Mean brachial artery blood pressure ($B_Pm$) was calculated according to the formula:

$$B_Pm = \frac{2}{3} \text{diastolic blood pressure}+\frac{1}{3} \text{systolic blood pressure}$$

and ocular perfusion pressure (PP) was derived using the following relation:

$$PP = \frac{2}{3} B_Pm - 10P$$

The relation between plasma and aqueous cGMP significantly differed in glaucoma and control patients (overall test of coincidence, $p = 0.003$; comparison of slopes, $p = 0.001$).
Ocular perfusion pressure

Perfusion pressure was calculated in 23 of the 38 glaucoma patients and in 22 of the 46 controls. Mean ocular PP was similar in POAGs (51.21 (5.66) mm Hg) and in normals (53.26 (6.40) mm Hg; p = 0.262).

Considering glaucomas and controls together, there was a linear, positive correlation between cGMP plasma levels and PP (n = 45, r = 0.379, p = 0.01), as well as between plasmatic NO2− and PP (n = 37, r = 0.339, p = 0.04). When the association of plasma cGMP and PP was explored in glaucoma patients and in controls, a positive correlation between the two variables was found in both groups (r = 0.249 and r = 0.423, respectively), but this relation did not attain statistical significance in POAG, whereas it was of borderline statistical significance in the control group (p = 0.050, fig 6). No statistically significant correlation was found between plasma NO2− levels and PP when glaucomas and controls were evaluated separately. No significant difference was found in the two groups of patients with respect to the association plasma cGMP with PP or plasma NO2− and PP.

DISCUSSION

In the present study, decreased concentrations of NO2− and cGMP were found in the plasma and aqueous humour of glaucoma patients. Our data on PP show that plasma NO correlates with PP, in that higher levels of cGMP and NO2− are associated with higher PP values.

The lower plasma levels of NO indicators in patients with POAG could reflect an impaired balance of endothelium derived mediators in glaucoma, as suggested by other evidence. 21–30

Decreased plasma cGMP seems to correlate with lower cGMP in the aqueous humour. As NO has a very short half life (2–30 s), and production of NO in the anterior chamber has been documented, 24–31 NO in aqueous humour should mainly derive from local formation. For this reason, the association found between plasma and aqueous humour cGMP is interesting, and suggests that a basic disorder in NO balance may act both systemically and locally. Our findings in the aqueous humour agree with those of other authors, 25 and with the report of decreased NOS reactivity in the aqueous outflow pathway of postmortem glaucomatous eyes. 33 Such alterations, together with pharmacological studies showing that NO donors reduce IOP elevating NO2− in the anterior chamber, 24 suggest that NO might be involved in the pathogenesis or regulation of IOP in POAG.

Higher IOP could alter NO concentration in the aqueous humour, but not in peripheral blood. Ocular antihypertensive medications such as β blockers could have an influence on NO levels in the aqueous humour and plasma. Although the β blocker propranolol inhibited the nitrite production evoked by a β adrenergic receptor agonist, it had no inhibitory effect either on NO formation induced by mediators other than β adrenergic agonists, or on basal production of NO. 24 Moreover, NOS reactivity of glaucomatous eyes which had never received any antiglaucomatous medications showed severe alterations, similar to those found in the treated eyes. 33 The effects of systemic β blockers on plasma NO levels are still unclear, even if some β blockers, such as nebivolol, are known to induce arterial and venous dilatation by greatly increasing cGMP. 35–36

Impaired NO formation can have a dual negative effect in glaucoma patients by acting on IOP and ocular blood supply, as shown by the relation between plasma cGMP or NO2− and PP. The correlation between the two parameters found in controls indicates that decreased NO levels are associated with lower PP. Nitric oxide could influence PP modulating...
both IOP and blood pressure. Lower PP is strongly associated with an increased prevalence of POAG. An impaired ONH autoregulation makes the glaucomatous optic nerve more sensitive to decreased PP. Recently, NO has been involved in choroidal autoregulation, making the glaucomatous optic nerve more sensitive to decreased PP. 19 Recently, NO has been involved in autoregulation makes the glaucomatous optic nerve more sensitive to decreased PP. 19

Figure 6 Linear regression between cGMP plasma levels and ocular perfusion pressure (PP).

ACKNOWLEDGEMENTS

Research winner, the Italian selection of the Merck Sharp & Dohme International Award 2002.

Authors’ affiliations

F Galassi, G Renieri, A Sodi, F Ucci, L Vannozzi, Department of Oto-Neuro-Ophthalmological Sciences, University of Florence, Eye Unit II, Via S Marta 24, 50139 Florence, Italy; fernando.galassi@unifi.it

E Masini, Department of Clinical and Preclinical Pharmacology, Florence, Italy

Correspondence to: Professor F Galassi, Department of Oto-Neuro-Ophthalmological Sciences, University of Florence, Eye Unit II, Via S Marta 24, 50139 Florence, Italy; fernando.galassi@unifi.it

Accepted 3 October 2003

REFERENCES

Nitric oxide proxies and ocular perfusion pressure in primary open angle glaucoma

F Galassi, G Renieri, A Sodi, F Ucci, L Vannozzi and E Masini

doi: 10.1136/bjo.2003.028357

Updated information and services can be found at:
http://bjo.bmj.com/content/88/6/757

These include:

References

This article cites 34 articles, 16 of which you can access for free at:
http://bjo.bmj.com/content/88/6/757#BIBL

Email alerting service

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections

Articles on similar topics can be found in the following collections

- Angle (1006)
- Glaucoma (988)
- Intraocular pressure (1002)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/