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# Brain antioxidant status in a high pressure–induced rat model of glaucoma

Sandra M. Ferreira,[1] Fabian Lerner,[2] Claudia G. Reides,[1] Ricardo Brunzini[1] and Susana F. Llesuy[1,3]

[1]General and Inorganic Chemistry Division, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina
[2]Fundation for the study of Glaucoma, Buenos Aires, Argentina
[3]Free Radicals Program (PRALIB-CONICET), Buenos Aires, Argentina

**ABSTRACT.**

***Purpose:*** **The goal of the present study is to establish the antioxidant status in the brain of a high pressure–induced rat model.**

***Methods:*** **Ocular hypertension was induced in rats ($n$ = 12) cauterizing two episcleral veins under a surgical microscope. A sham procedure ($n$ = 12) was performed in the control group. The markers evaluated in the brain 7 days after surgery were as follows: spontaneous chemiluminescence, protein carbonylation, nitrite concentration, total reactive antioxidant potential (TRAP), ascorbic acid, glutathione, vitamin E and activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase.**

***Results:*** **Chemiluminescence in glaucoma was 55% higher than in controls (393 ± 20 cpm/mg protein, $p < 0.001$). Protein carbonylation in glaucoma was 93% higher than in controls (1.15 ± 0.18 nmol/mg protein, $p < 0.001$). Nitrite concentration was 5.30 ± 0.25 μM for glaucoma (controls 4.41 ± 0.24 μM, $p < 0.05$). Total reactive antioxidant potential decreased by 42% in glaucoma (controls 153 ± 14 μM Trolox, $p < 0.001$). Ascorbic acid was 67 ± 26 μM for glaucoma (controls 275 ± 22 μM, $p < 0.001$). Vitamin E was 0.58 ± 0.05 μmol/g organ for glaucoma (controls 1.10 ± 0.06 μmol/g organ, $p < 0.01$). Glutathione was 1.98 ± 0.13 μmol/g organ for glaucoma (controls 8.19 ± 0.71 μmol/g organ, $p < 0.001$). Superoxide dismutase and GPx were increased in glaucoma by 42 and 59%, respectively ($p < 0.05$).**

***Conclusions:*** **Reactive oxygen and nitrogen species were increased in glaucoma, the increase in chemiluminescence, protein carbonylation and nitrite levels could be evidenced by this situation. The decrease in nonenzymatic antioxidants and a compensatory increase in SOD and GPx activity may have been a consequence of an increase in oxidative processes.**

**Key words:** antioxidants – glaucoma – high pressure – induced rat model – oxidative damage – oxidative stress

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## Introduction

Glaucoma is the second leading cause for blindness worldwide, and the first irreversible ocular disease (Quigley 1996). It is estimated that for the year 2020, there will be 79.6 million people with the disease worldwide, and 74% will suffer open-angle glaucoma (Quigley & Broman 2006). Primary open-angle glaucoma (POAG) is a multifactorial optic neuropathy in which there is a characteristic acquired loss of retinal ganglion cells and atrophy of the optic nerve (Landers 1982). Although elevated intraocular pressure (IOP) is the most important known risk factor (Epstein et al. 1997), other factors have been suggested to contribute to the glaucomatous optic neuropathy. Proposed mechanisms include ischaemia (Cioffi 2005), obstruction of axoplasmic flow (Anderson & Hendrickson 1974), deprivation of one or more trophic factors (Quigley et al. 1995), excitoxicity (Vorwerk et al. 1997) and oxidative stress (Aslan et al. 2008).

Oxidative stress can be defined as an increase over physiological values in the intracellular concentrations of reactive oxygen species (ROS) (Halliwell & Gutteridge 1989). This situation is reflected by changes in the antioxidant defences that can increase, as a protective response, or deplete owing to the ROS action (Sies 1985).

Evidence of oxidative and nitrative processes was found in several ocular disorders in terms of activity of antioxidant enzymes, levels of low molecular weight antioxidants and markers of lipid peroxidation (Izzotti et al. 2003; Kumar & Agarwal 2007; Ferreira et al. 2010). Moreover, it has been reported that nitric oxide (NO) may be an important mediator in retinal ganglion cells′ death in glaucoma (Kumar & Agarwal 2007).

Previous studies of our group have reported the occurrence of oxidative stress in an experimental rat glaucoma model. We have shown that there was a decrease in nonenzymatic antioxidant and an increase in the activities of antioxidant enzymes. This situation may overcome the ability of cells to resist oxidative damage evidence by the increased levels of lipid peroxidation products and *in vivo* chemiluminescence (Ferreira et al. 2010).

Although it is usually considered solely as an eye disease, glaucoma also damages other structures in the brain, including the lateral geniculate nucleus (LGN) of the thalamus and the primary visual cortex. Chronic ocular hypertension induces dendrite pathology in the LGN of the brain. These changes have been observed both in experimental animals (Weber et al. 2000; Yücel et al. 2000; Crawford et al. 2001) and humans (Chaturvedi et al. 1993; Gupta et al. 2006).

Chronic ocular hypertension monkey model induces dendrite pathology in the LGN of the brain. The nitrotyrosine positivity in endothelial cells of blood vessels throughout the LGN layers showed that the vascular endothelial cells are targets for oxidative stress–induced damage (Luthra et al. 2005).

Transneuronal degeneration has been demonstrated in the central nervous system for different diseases, including Alzheimer and glaucoma (Su et al. 1997; Yücel et al. 2001). Neuron death and degeneration in glaucoma have been reported both at the LGN and cortical levels (Crawford et al. 2001; Gupta et al. 2006; Tezel 2006).

The purpose of this study was to measure the changes in oxidative stress markers to evaluate the antioxidant status of primary visual targets in the brain of a high pressure–induced rat model of glaucoma. The occurrence of oxidative stress was assessed by the measurement of the following markers: spontaneous chemiluminescence (CL), protein carbonylation (PC), nitrite concentrations (NC), total reactive antioxidant potential (TRAP), ascorbic acid (AA), vitamin E (VE), glutathione (GSH) levels and the activities of antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx).

# Methods

### Experimental glaucoma model

A chronic ocular hypertension model was used following episcleral venous occlusion in rats (Shareef et al. 1995). Female Wistar rats (3 months of age) weighting 250–300 g were operated under a microscope with a coaxial light. Animals were anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (0.5 mg/kg) administrated intraperitoneally.

A specially designed small lid speculum was used to retract the eyelids. Vannas scissors and a nontoothed forceps were used to open the conjunctiva and expose the limbal veins.

A cyclodyalisis spatula was utilized to gently lift the vein from the underlying sclera, and an ophthalmic cautery was employed to cauterize the vein. Special care was taken to avoid damage of the sclera. Two of the large veins of the left eye were cauterized using this method for the glaucoma group ($n$ = 12). Retraction without bleeding was noted after cauterization.

A sham operation without cauterizing the vessels was performed in the left eye of the control group ($n$ = 12). These sham eyes were maintained under the operating microscope to match the time the experimental animals were subjected to the microscope light.

The rats were housed in standard animal rooms in a 12-hr light/dark cycle and were fed with free access to food and water under controlled conditions of temperature (21 ± 2°C) and humidity. Seven days after the surgery, the animals were anesthetized as previously described and then killed by decapitation. The brains were removed from the skulls carefully. All the procedures and handling were performed according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

### Intraocular pressure assessment

The IOP was measured with a Tonopen XL (Mentor, Norwell, MA, USA) tonometer. All IOP determinations were performed in unsedated rats as previously described (Moore et al. 1995). Measurements were recorded from animals, which were placed in special rodent containers. The eyelids were opened with care to prevent the eye compression, and the Tonopen was applied to the cornea. Fifteen readings were taken and averaged for each record. The instrument was applied firmly and perpendicular to the cornea to obtain the readings. A masked observer performed all the readings. IOP measurements were performed before and after the procedure and at 7 days. Results are expressed as mmHg.

### Preparation of brain homogenates

The animals were anesthetized as previously described; then, killed by decapitation; and the brains were removed from the skulls carefully. The brain was immediately cooled in ice. Each brain was dissected, the LGN and the primary visual cortex were separated from it and homogenized in 120 mM KCl, 30 mM phosphate buffer (pH 7.40). Tissue samples were homogenized in five times their weight of buffer. Rat brains homogenates were centrifuged at 2600 ***g*** for 15 min at 4°C to discard nuclei and cell debris. The supernatant fraction obtained from this procedure was called brain homogenate.

### Spontaneous chemiluminescence

The chemiluminescence of brain homogenates was measured in a Packard liquid scintillation counter, at room temperature, in the out-of-coincidence model (Lissi et al. 1988). The emission was expressed in counts per minute. The reaction medium consisted of 120 mM KCl, 1 mM EDTA and 30 mM phosphate buffer (pH 7.40). Results are expressed as cpm/mg of protein.

### Protein carbonylation

Protein carbonyl groups were detected with 2,4-dinitrophenylhydrazine (DNPH) 10 mM, which leads to the formation of a stable 2,4-dinitrophenylhydrazone product (DNP) that is soluble in 6 M

guanidine. The DNP absorbs ultraviolet light so that the total carbonyl content of a protein can be quantified by a spectrophotometric assay at 370 nm (Levine et al. 1990). Results are expressed as nmol/mg of protein.

### Nitrite concentration

The nitrite concentration was measured using a spectrophotometric method based on the Griess reaction. Samples were mixed with 1% P/V sulphanilamide and 0.1% P/V naphthyl-ethylenediamine, which were allowed to react at room temperature for 10 min. The nitrite concentration was determined by measuring the absorbance at 550 nm in comparison with standard solutions of sodium nitrite (Ding et al. 1988). Results are expressed as $\mu$M.

### Total reactive antioxidant potential

Total reactive antioxidant potential was measured by chemiluminescence in a Luminoskan V 1.2-0 liquid scintillation counter (Labsystems Research Systems Division, Finland). The reaction medium consisted of 20 mM 2,2-Azobis (2- amidinopropane) (ABAP) and 40 $\mu$M luminol. The system was calibrated with different concentrations (0.25–0.50 $\mu$M) of Trolox (vitamin E analogue). A comparison of the induction time after the addition of Trolox and the brain homogenate allows calculation of TRAP as the equivalent of Trolox concentration necessary to produce the same induction time (Lissi et al. 1992; Ferreira et al. 2004). Results are expressed as $\mu$M Trolox.

### Ascorbic acid levels

Ascorbic acid concentration was evaluated spectrophotometrically according to the method described by Kyaw (1978). Samples were treated with 1.36 M phosphotungstic acid and incubated at room temperature for 30 min. After centrifugation, the absorbance of the supernatant was measured at 700 nm. A standard curve was established with a set of serial dilutions of AA. Results are expressed as $\mu$M.

### Vitamin E levels

Vitamin E was measured by chemiluminescence in a Luminoskan V 1.2-0 liquid scintillation counter. Samples were mixed with sodium dodecyl sulphate 0.02 M, n-hexane and ethanol. The organic phase was dried under a nitrogen flux at room temperature and resuspended in ethanol. An aliquot of this solution was employed in the evaluation of the total charge of lipid-soluble antioxidants. The reaction mixture contains 200 mM ABAP, 2 mM luminol and dodecyl trimethyl ammonium bromide in buffer glycine pH 9.40. This solution was allowed to stand for a few minutes ($I_0$). The incorporation of the samples produces a decrease in the intensity ($I$). The relation between $I_0/I$ is related to the concentration of lipid-soluble antioxidants. A standard curve was established with a set of serial dilutions of $\alpha$-tocopherol (Escobar et al. 1997). Results are expressed as $\mu$mol/g organ.

### Glutathione levels

Total glutathione was determined in brain homogenates; the reaction medium consisted of 100 mM phosphate-buffered pH 7.00, 6 mM 5, 5′-dithiobis (2 nitrobenzoic acid) (DTNB), 10 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH) and 6 U/ml glutathione reductase in a kinetic assay at 412 nm. Proteins were eliminated through the addition of 0.5 M perchloric acid. Glutathione concentration is expressed in $\mu$mol/g organ (Akerboom & Sies 1981).

### Superoxide dismutase activity

Superoxide dismutase activity was determined in brain homogenates by following the inhibition of the rate of autocatalytic adenochrome formation at 480 nm with a Hitachi U-2000 (Hitachi, Tokyo, Japan). The reaction medium contained 1 mM epinephrine and 50 mM glycine – NaOH (pH 10.20). The enzyme activity is expressed as U SOD/mg of protein. One unit is defined as the amount of enzyme that inhibits the rate of adrenochrome formation by 50% (Misra & Fridovich 1972).

### Catalase levels

Catalase activity was determined by measuring the decrease in absorption at 240 nm. The reaction medium consisted of 100 mM phosphate buffer (pH 7.20) and 10 mM hydrogen peroxide. The results are expressed as CAT content in pmol/mg of protein (Chance et al. 1979).

### Glutathione peroxidase activity

Glutathione peroxidase activity was determined by following reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidation at 340 nm. The reaction medium consisted of 100 mM phosphate buffer (pH 7.50) in presence of 10 $\mu$M reduced glutathione, 6 U/ml glutathione reductase and 10 mM *tert*-butyl hydroperoxide (Flohé & Günzler 1984). Results are expressed as $\mu$mol/mg protein/min

### Laboratory determinations

All laboratory determinations were done in a masked fashion.

### Chemicals

All the chemicals were purchased from Sigma Aldrich Chemical (St Louis, MO, USA).

### Data and statistical analysis

Statistical calculations were performed with the GRAPHPAD INSTAT statistical package for Windows Graph Pad Software Inc. La Jolla, CA, USA. Data are expressed as mean value ± SEM (standard error of the mean). Statistical significance of the differences between glaucoma and control groups was calculated by the two-tailed unpaired Student's *t*-test, and a probability value of p smaller than 0.05 indicated a statistically significant difference.

## Results

Ocular hypertension was induced in Wistar rats ($n$ = 12) by cauterizing two episcleral veins under a surgical microscope (glaucoma group). A sham procedure ($n$ = 12) was performed in the control group. IOP increased in all glaucoma group animals. IOP was higher in the experimental group at day 7 (24 ± 3 mmHg) when compared to the control group (14 ± 2 mmHg $p < 0.001$) and when compared to the preoperative IOP (13 ± 2 mmHg $p < 0.001$). No difference was found in the control group between pre- and postoperative IOP (Fig. 1).

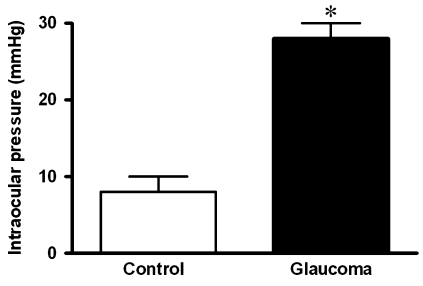


**Fig. 1.** Intraocular pressure in control and glaucoma animals at 7 days after surgery. The values are represented as mean ± SEM for 12 animals in both groups (*p < 0.001). Statistical significance of the differences between glaucoma and control groups was calculated by the two-tailed unpaired Student's *t*-test.

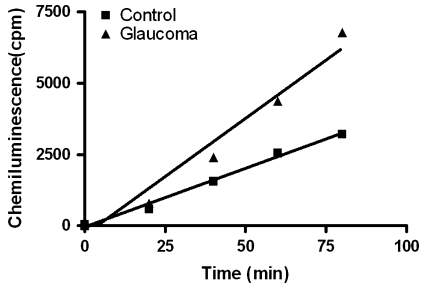


**Fig. 2.** Typical time profiles of chemiluminescence from one glaucoma (*r*: 0.9833; p < 0.001) and one control (*r*: 0.9965; p < 0.001) brain homogenates.

Figure 2 shows a representative result of the chemiluminescence kinetics of brain homogenates from a glaucoma rat and a control one. Both profiles exhibit a significant difference in the rate of luminescence increase. This increase in light intensity is almost linear throughout the entire time period in both groups (glaucoma *r*: 0.9833 p < 0.001 and control *r*: 0.9965 p < 0.001). The photoemission of brain homogenates in glaucoma is higher than in the control group at any considered time period.

The results of oxidative stress markers are shown in Table 1. Spontaneous brain chemiluminescence at 60 min for glaucoma group was 55% higher than in the control group (393 ± 20 cpm/mg protein p < 0.001). Protein carbonylation was increased a 93% in glaucomatous brains when compared to controls (1.15 ± 0.18 nmol/mg protein p < 0.001). Nitrite concentration in the glaucoma group was 20% higher than in the control group (4.41 ± 0.24 $\mu$M p < 0.05).

**Table 1.** Oxidative stress markers of brain homogenates in control and glaucoma animals.

| | Groups | |
|---|---|---|
| Oxidative stress markers | Control | Glaucoma |
| Chemiluminiscence (cpm/mg protein) | 393 ± 20 | 609 ± 57[a] |
| Protein carbonyls (nmol/mg protein) | 1.15 ± 0.18 | 2.22 ± 0.31[a] |
| Nitrite concentration ($\mu$M) | 4.41 ± 0.24 | 5.30 ± 0.25[b] |

The values are represented as mean ± SEM for 12 animals in both groups. Statistical significance of the differences between glaucoma and control groups was calculated by the two-tailed unpaired Student's *t*-test.
[a] p < 0.001, [b] p < 0.05.


**Fig. 3.** Total reactive antioxidant potential of brain homogenates in control and glaucoma groups. The values are represented as mean ± SEM for 12 animals in both groups (*p < 0.001). Statistical significance of the differences between glaucoma and control groups was calculated by the two-tailed unpaired Student's *t*-test.

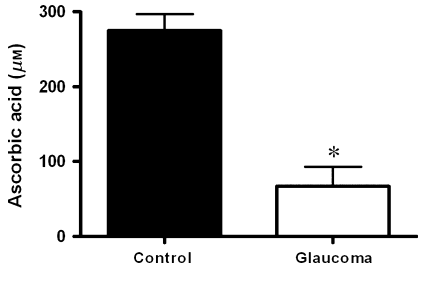


**Fig. 4.** Ascorbic acid concentration of brain homogenates in control and glaucoma groups. The values are represented as mean ± SEM for 12 animals in both groups (*p < 0.001). Statistical significance of the differences between glaucoma and control groups was calculated by the two-tailed unpaired Student's *t*-test.

The results for nonenzymatic antioxidants are shown from Figs 3 to 6. TRAP levels were decreased in glaucoma samples compared with controls (Fig. 3). TRAP results were 90 ± 12 $\mu$M Trolox for the glaucoma group and 153 ± 14 $\mu$M Trolox for the control group (p < 0.001). Ascorbic acid levels were 67 ± 26 and 275 ± 22 $\mu$M (p < 0.001), respectively, for glaucoma and control samples (Fig. 4).

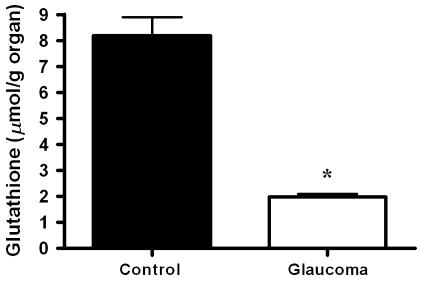


**Fig. 5.** Glutathione levels of brain homogenates in control and glaucoma groups. The values are represented as mean ± SEM for 12 animals in both groups (*p < 0.001). Statistical significance of the differences between glaucoma and control groups was calculated by the two-tailed unpaired Student's *t*-test.

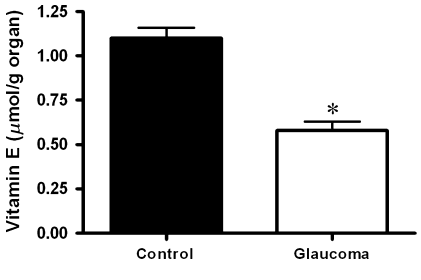


**Fig. 6.** Vitamin E concentration of brain homogenates in control and glaucoma groups. The values are represented as mean ± SEM for 12 animals in both groups (*p < 0.001). Statistical significance of the differences between glaucoma and control groups was calculated by the two-tailed unpaired Student's *t*-test.

Glutathione concentration was 1.98 ± 0.13 $\mu$mol/g organ for the glaucoma group and 8.19 ± 0.71 $\mu$mol/g organ for the control group, p < 0.001 (Fig. 5).

Vitamin E levels were 0.58 ± 0.05 $\mu$mol/g organ for the glaucoma group (CG 1.10 ± 0.06 $\mu$mol/g organ p < 0.01).

The activities of antioxidant enzymes are displayed in Table 2. SOD and GPX activities were increased in glaucoma

**Table 2.** Activities of antioxidant enzymes of brain homogenates in control and glaucoma groups.

| Antioxidant enzymes | Groups | |
|---|---|---|
| | Control | Glaucoma |
| Superoxide dismutase (U/mg protein) | 2.51 ± 0.19 | 3.55 ± 0.37* |
| Glutathione peroxidase ($\mu$mol/mg protein/min) | 0.042 ± 0.003 | 0.067 ± 0.008* |
| Catalase (pmol/mg protein) | 0.077 ± 0.01 | 0.078 ± 0.01 |

The values are represented as mean ± SEM for 12 animals in both groups. Statistical significance of the differences between glaucoma and control groups was calculated by the two-tailed unpaired Student's *t*-test.
* $p < 0.05$.

samples (3.55 ± 0.37 U/mg protein and 0.067 ± 0.008 $\mu$mol/mg protein/min, respectively) when compared to controls (2.51 ± 0.19 U/mg protein $p < 0.05$ and 0.042 ± 0.003 $\mu$mol/mg protein/min $p < 0.05$ for SOD and GPX, respectively). There were no statistical differences between both groups for CAT levels (0.078 ± 0.01 and 0.077 ± 0.01 pmol/mg protein for glaucoma and controls, respectively).

# Discussion

Different reports have confirmed that the model of IOP elevation through the cauterization of episcleral vessels is a useful animal model for the study of glaucoma (Sawada & Neufeld 1999; Ferreira et al. 2010). Persistent elevation of IOP with optic nerve cupping and loss of retinal ganglion cells provides a reproducible animal model for studying glaucomatous damage (Garcia Valenzuela et al. 1995; Morrison et al. 1997). While elevated IOP is the principal risk factor for glaucoma, a significant number of glaucoma patients never exhibit elevated IOP (Weinreb & Khaw 2004; Flammer & Mozaffarieh 2007).

Glaucoma is usually considered as an eye disease; however, most of the axons of the retinal ganglion cells are extraocular and remain intraorbital and intracranial. Axons project to LGN of the thalamus, and the axons of relay LGN neurons form the optic radiations, which project to eye-specific columns in the primary visual cortex (Gupta & Yücel 2001).

Previous studies of our group have demonstrated the time course changes of oxidative stress markers in the retina of a high pressure–induced rat model of glaucoma (Ferreira et al. 2010). We analysed nitrite levels and markers of lipid peroxidation at 7, 15, 30, 45 and 60 days after the surgery. As an increase in lipid peroxidation and nitrite levels were observed since 7 days, we decided to kill the animals at this time.

The central nervous system is particularly vulnerable to oxidative stress owing to its high lipid content and elevated metabolic rate based on very high levels of oxygen utilization and synthesis of ATP. This is in accordance with the results of the present study. Oxidative stress markers were significantly elevated in primary visual targets in the brain of glaucoma rats. Spontaneous chemiluminescence was 54% higher in glaucoma samples than control samples, showing an increase in lipid peroxidation.

Proteins are important targets of oxidative damage mediated by ROS. Oxidized proteins that are assessed by protein carbonylation were 93% increased in glaucoma samples compared with controls. Oxidation has been described as one of the most important mechanisms of brain protein damage and dysfunction in several neurodegenerative disorders, including Alzheimer's disease (Smith et al. 1991; Tezel et al. 2005). Oxidative modification of important proteins has been shown in the retina as a consequence of free radicals generation in the glaucoma rat model (Tezel et al. 2005). Our study shows that this process also occurs early in primary visual targets in the brain.

A reduction in the antioxidant capacity of tissues may be due to an increase in oxidative processes. Among the methods used to determine the antioxidant capacity of a biological sample, TRAP is one of the most widely used (Lissi et al. 1995; Ferreira et al. 2004). We found a 41% decrease in TRAP levels of glaucoma samples compared with controls. These results indicated a significant reduction in the concentrations of water-soluble antioxidants, such as glutathione and AA. This decrease may occur owing to oxidative stress in the primary visual targets of the glaucoma brains, rendering the organ more susceptible to the damage associated with ROS production. There was a 76% decrease in AA levels of glaucoma homogenates compared with controls. These findings seem to be significant because AA is essential in cells for its antioxidant capacity and its role in regenerating vitamin E and glutathione (Packer et al. 1979). The changes measured in our work in nonenzymatic antioxidants evaluated by TRAP are consistent with the decay observed in AA and glutathione levels.

In such conditions of deficient antioxidant nonenzymatic defences, an increase in the activities of the antioxidant enzymes could be expected as a tissue-adaptative response. According to this, the experimental group presented a 59% higher GPX and a 42% higher SOD activity when compared to the control group. SOD catalyses the reaction of superoxide anion to produce hydrogen peroxide, this molecule is an essential component of several signal transduction pathways (Trachootham et al. 2008) and when its levels are over physiological values, it is removed by two antioxidant enzymes: CAT and GPX. Alterations in GPX activity might have important consequences on the steady-state concentration $H_2O_2$. As GPX removes hydrogen peroxide using glutathione as a cofactor, this might contribute to its depletion. Glutathione is involved in AA metabolism, and its depletion produces ascorbyl radicals that cannot be regenerated to AA (Halliwell & Gutteridge 1989). This water-soluble antioxidant is involved in vitamin E recycling, and its depletion produces tocopheryl radicals. Vitamin E protects the cells membranes against oxidative damage by scavenging peroxyl radicals (Niki 1996). A reduction in the levels of this lipid-soluble antioxidant in the brain could be related to its reaction with free radicals generated. The increased observed in chemiluminescence may be due to the formation of hydroxyl radical. This radical is generated *in vivo* from the combination of iron or copper ions,

hydrogen peroxide and a reducing agent as ascorbate by Fenton reaction. The high reactivity of hydroxyl radical makes it an unselective oxidant and reacts with many biomolecules at rates approaching the diffusion limit.

Cells survival depends on the detoxification of $H_2O_2$ and the inhibition of CAT could increase hydrogen peroxide levels, which is not compensated by the increase in GPX activity. No significant changes were found in CAT levels, and this may be due to NO inhibition of the CAT by the binding of it to the haem group of the enzyme (Brown 1995). Nitric oxide competes with hydrogen peroxide for the union to the complex I of the CAT; when this enzyme is inhibited, hydrogen peroxide has to be metabolized by GPX.

Nitric oxide would react with hydroxyl radical to generate nitrite or with superoxide anion to produce peroxynitrite, a powerful oxidant. The increase in NO could be evidenced by the increase in its final metabolites, nitrite (Hogg & Kalyanaraman 1999). The capacity of nitrite to modulate multiple signalling pathways is mediated by nitrosylation ad or thiol-nitrosation. Thus, nitrite may be the mediator that determines the levels of these products (Van Faassen et al. 2007).

Oxidative stress markers have been documented in the aqueous humour of primary open-angle glaucoma patients (Ferreira et al. 2004) and in patients with glaucoma associated with exfoliation syndrome (Ferreira et al. 2009). Furthermore, markers of oxidative stress such as protein carbonyl formation and lipid peroxidation products have been found in the retina of experimental models of ocular hypertension (Ko et al. 2005; Tezel et al. 2005).

Our results are consistent with this mechanism, as an increase in the activity of GPX and a decrease in TRAP and AA concentration were measured, providing evidence for a role of free radical–induced oxidative damage in the experimental brains. We suggest that this situation may have been owing to a defective redox cycling of reduced/oxidized glutathione.

Our results show, for the first time, a significant decrease in the nonenzymatic defence, a significant increase in the activities of enzymatic defences and a significant enhancement in the markers of oxidative damage in primary visual targets in the brain of experimental glaucoma model. The generation of reactive oxygen and nitrogen species evaluated by chemiluminescence, nitrite concentration and protein carbonylation are consistent with the existence of oxidative stress. Reactive oxygen and nitrogen species are involved in many neuropathology disorders (Smith et al. 1991; Tezel et al. 2005).

Based on our findings, we suggest that the oxidative stress found in primary visual targets in the brain of glaucoma rats in the early period after IOP elevation may possibly act as a risk factor for neurodegeneration in glaucoma. Therapeutic strategies to stop disease progression in glaucoma should also consider central neural degeneration beyond the retina and the optic nerve. Treatment interventions to reduce *in vivo* oxidative stress may be important in patients with this disease. Future studies will improve our knowledge on the mechanisms of damage in glaucoma not only in the eye, but also at the level of the central nervous system, and thus devise more effective treatments, in addition to IOP reduction.

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*Correspondence:*
Sandra Maria Ferreira
General and Inorganic Chemistry Division
School of Pharmacy and Biochemistry
University of Buenos Aires, Junin 956
(1113) Buenos Aires
Argentina
Tel/Fax: 54 11 4964 8249
Email: smferrer@ffyb.uba.ar