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Changes in the redox status of the brain in an experimental glaucoma model

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ABSTRACT: The purpose of this study was to evaluate the redox status changes of primary visual targets in the rat brain of a high pressure-induced glaucoma model. The animal model consisted of inducing ocular hypertension by cauterizing two episcleral veins on the left eye. The markers of oxidative damage and the oxidative balance evaluated in the brain seven days postoperative were: nitrites concentration, levels of non-enzymatic antioxidants and antioxidant enzymes activity.

The increase in the nitrite content, which could be the result of the enhancement in the production of nitrogen species, and in the activity of NADPH oxidase in the glaucoma group could lead to an increase on lipid and protein damage.

The decrease on the non-enzymatic antioxidants and the compensatory increase of the superoxide dismutase and glutathione peroxidase activities could be a consequence of the increase of oxidative processes. The decrease in the activity of glutathione reductase leads to a decrease in the recycling of thiol groups.

We suggest that oxidative stress can possibly acts as a risk factor for neurodegeneration in the brain. Therapeutic strategies to stop the progression of the disease in glaucoma should also be considered the central neuronal degeneration beyond the retina and the optic nerve.

The central nervous system is particularly vulnerable to oxidative stress due to its high lipid content and elevated metabolic rate based on high levels of oxygen utilization and synthesis of ATP. Glaucoma is a multifactorial optic neuropathy in which there is a characteristic acquired loss of retinal ganglion cells and atrophy of the optic nerve. Although elevated intraocular pressure (IOP) is the most important known risk factor, other factors have been suggested to contribute to the glaucomatous optic neuropathy. Proposed mechanisms include ischemia (Cioffi, 2005), excitoxicity (Vorwerk *et al.,* 1997), obstruction of axoplasmic flow (Anderson and Hendrickson, 1974) and oxidative stress (Ferreira *et al.,* 2010, Ferreira *et al.,* 2011).

Numerous authors have documented the existence of oxidative and nitrosative stress in glaucoma, either in terms of activity of antioxidant enzymes, levels of antioxidants and lipid peroxidation markers in different ocular structures (Izzotti *et al.,* 2003, Kumar and Agarwal, 2007, Ferreira *et al,* 2013). On the other hand, nitric oxide can be an important mediator in the death of retinal ganglion cells in glaucoma (Ko e*t al.,* 2005).

Although it is usually considered solely as an eye disease, glaucoma also damages other structures in the brain, including the lateral geniculate nucleus of the thalamus and the primary visual cortex. Transneuronal degeneration has been demonstrated in the central nervous system for different diseases, including Alzheimer and glaucoma (Gupta *et al.,* 2006).

The purpose of this study was to evaluate the redox status changes of primary visual targets in the brain of a high pressure-induced rat model of glaucoma. The status of the antioxidant defense was determined by measuring not only the content of ascorbic acid, glutathione and vitamin E, but also the activities of the enzymes NADPH

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oxidase, superoxide dismutase, glutathione reductase and glutathione peroxidase. Besides the nitrite content was evaluated in order to estimate nitrogen species levels.

A chronic ocular hypertension model was used following episcleral venous occlusion in rats (Shareef *et al.,* 1995). Female Wistar rats (3 months of age; *n*=12) weighing 250-300 g were operated under a microscope with a coaxial light. Persistent elevation of IOP with optic nerve cupping and loss of retinal ganglion cells provides a reproducible animal model for studying glaucomatous damage. The animals were sacrificed by decapitation seven days after surgery, and the brains were removed and placed on ice. The lateral geniculate nucleus and the primary visual cortex were dissected out from each brain, pooled and homogenized in phosphate buffer 30 mM and KCl 120 mM, pH=7.40 (Ferreira *et al.,* 2013).

Nitrite concentration was significantly increased in glaucomatous rats (control, 4.41 ± 0.24 µM; glaucoma, 5.30 ± 0.25 µM). This supports a possible increase in other nitrogen species, because nitrite is a final metabolite of several metabolic pathways. Also in that study, the ascorbic acid content (control, 275 ± 22 µM; glaucoma, 67 ± 26 µM; $p < 0.001$) and the glutathione levels were significantly decreased in glaucomatous rats (control. 8.19 ± 0.71 µmol/ g of tissue; glaucoma, 1.98 ± 0.13 µmol/g; * $p < 0.001$). These results indicated a significant reduction in the concentrations of water-soluble antioxidants that may occur due to oxidative stress in the primary visual targets of the glaucoma brains, rendering the organ more susceptible to the damage associated with reactive oxygen species production. Also, the vitamin E concentration was decreased in glaucomatous rats (control group 1.10 ± 0.06 µmol/ g of tissue; glaucoma, 0.58 ± 0.05 µmol/ g of tissue; $p < 0.01$). A reduction in the levels of this lipid-soluble antioxidant in the brain could be related to its reaction with the generated free radicals. Concomitantly, the activities of both superoxide dismutase (SOD) and glutathione peroxidase (GPx) were significantly increased ($p < 0.05$) in glaucomatous rats by 42 % y 59 %, respectively (as compared with the control groups: SOD 2.51 ± 0.19 U/ mg of protein and GPx 0.042 ± 0.003 µmol/ min. mg of protein. SOD catalyzes the reaction of superoxide anion to produce hydrogen peroxide. When the levels of hydrogen peroxide are over physiological values, catalase and GPx remove it, so the increase in GPx activity might have important consequence on the steady-state concentration of hydrogen peroxide. Moreover, this might contribute to the decrease in glutathione as GPx removes hydrogen peroxide using glutathione as a cofactor. The activity of glutathione reductase (GR) decreased by 42 % (p <0.05) (control, 10.2 ± 0.4 nmol/ min.mg of protein. GR is involved in the recycling of glutathione so the decrease in glutathione levels could also be related to the decay of its activity. The NADPH oxidase is significantly increased (p<0.001) by 148 % (control 0.046 ± 0.006 U/ mg of protein). The increase in NADPH oxidase activity leads to a rise in the steady state concentration of the superoxide anion that is metabolized by superoxide dismutase, whose activity is increased, to produce hydrogen peroxide, which then generates hydroxyl radical via the Fenton, Haber -Weiss reaction.

In summary, a significant decrease in the non-enzymatic defense as well as a significant increase in the activities of antioxidant enzymes and in the markers of oxidative damage in primary visual targets in the brain of experimental glaucoma model were measured. The generation of active oxygen and nitrogen species were increased in the brain and this

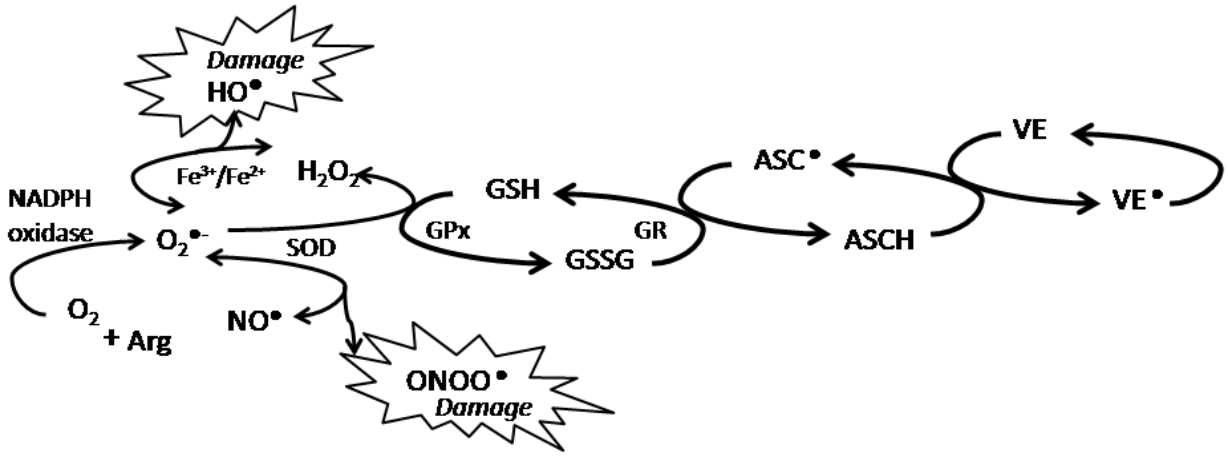

FIGURE 1. Proposed mechanism of brain damage for the changes in the redox status in experimental glaucoma model.

may be supported by the increase in NADPH oxidase activity and the increase in the nitrite concentration.

The possible increase of both oxygen and nitrogen in the glaucoma model may produce lipid and protein damage. The decrease on the non-enzymatic antioxidants and the compensatory increase of the superoxide dismutase and glutathione peroxidase activities may have been a consequence of the increase of the oxidative processes. A decrease in the activities of glutathione reductase leads to a decrease in the recycling of thiol groups (Fig. 1).

Based on our findings, we suggest that the oxidative stress found in primary visual targets in the brain of glaucomatous 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 considered, i.e., central neural degeneration beyond the retina and the optic nerve. Treatment interventions to reduce 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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