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Oxidative damage of brain structures in a glaucoma rat model and the protective role of lipoic acid

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Abstract

Evidence of oxidative process was found in glaucoma brain. The purpose was to evaluate the role of lipoic acid (LA) in oxidative damage of geniculate nucleus (GN) and visual cortex (VC) in glaucoma model.
Four groups of Wistar rats (n=20) were used: Glaucoma(G) rats operated by cauterized episcleral veins, glaucoma treated with LA 100 mg/kg(LG), Control(C) received a sham procedure and Control treated with LA 100 mg/kg (LC). At seven days brain were removed. Thioredoxin reductase (TRxR), glutathione reductase(GR), superoxide dismutase(SOD), protein oxidation(PO), lipid damage(TBARS) and glutathione(GSH) were evaluated.
Comparing LG to G: TRxR increased 52% in GN(7.6 ±0.4 nmol/min.mg protein $p<0.01$) and 26% in VC(9.9±1.0 nmol/min.mg protein $p<0.05$), GR increased 82% in GN(8.2±1.2nmol/min.mg protein $p<0.01$) and 300% in VC (5.1±1.3nmol/min.mg protein $p<0.001$), SOD increased 23% in GN(18.0±1.1 U/mg protein $p<0.05$) and 80% in VC(6.0±0.4 U/mg protein $p<0.001$), PO diminished 52% in GN(22.9±2.3nmol/mg protein $p<0.05$) and 58% in VC (9.3±1.3 nmol/mg protein $p<0.05$), TBARS diminished 25% in GN(4.8±0.4 nmol/mg protein $p<0.05$) and 36% in VC(5.3±0.6nmol/mg protein $p<0.05$), GSH increased 42%(0.20±0.04µmol/g $p<0.05$) in GN and 73% (0.41±0.03µmol/g $p<0.01$) in VC.
The effect against lipid and protein damage and the improvement in GSH recycling support that LA could be used in glaucoma.

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Introduction

Glaucoma is a disease characterized by a specific pattern of optic nerve head damage and visual field loss that, if not controlled, can lead patient to blindness. It is currently considered a multifactorial optic neuropathy that presents loss of retinal ganglion cells and optic nerve atrophy. Despite the fact that 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, excitoxicity, obstruction of axoplasmic flow and oxidative stress (Ferreira *et al*., 2010).

Our group 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 (Ferreira *et al*, 2013). 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.

The development of IOP independent treatments for glaucoma based on neuroprotection could be a different approach to improve the management of glaucoma. Since glaucoma is partially mediated by an increase in reactive oxygen species, antioxidant therapies to reduce in vivo oxidative stress may be important in patients with this disease. α-Lipoic acid (LA) has been used to attenuate oxidative damage in retinopathy and in the treatment of neurodegeneration (Inman *et al*, 2013) for its ability to cross the brain barrier and its antioxidant properties. The aims of this work are to discuss the changes that occur in redox homeostasis in lateral geniculate nucleus (GN) and visual cortex (VC) of rats subjected to an experimental glaucoma model and the modifications found in evaluated markers when LA is administered.

Material & Methods

Experimental glaucoma model: Ten animals received an episcleral venous occlusion (glaucoma) (Shareef et al., 1995), while other ten received a sham procedure (control). Rats were divided in four groups (n=5 each group), control received a sham procedure (C), control treated with LA 100 mg/kg i.p. (LC), glaucoma (G), glaucoma treated with LA 100 mg/kg i.p. (LG). 7 days after the surgery rats were euthanized and brains were removed.

Homogenates: GN and VC were separated from brain and then homogenized in five times their weight of 120 mM KCl, 30 mM phosphate buffer (pH 7.40) at 2600 g for 15 min at 4 °C. (Ferreira et al., 2013).

Thioredoxin reductase (TRxR): was evaluated spectrophotometrically with DTNB and NADPH at 412 nm. Results were expressed as nmol/ min.mg protein (Holmgren & Bjornstedt, 1995).

***Glutathione reductase (GR)*:** was determined by following NADPH oxidation at 340 nm. The reaction medium consisted of buffer Tris 100 mM, EDTA 10 mM, NADPH 10 mM, oxidized glutathione 25 mM. Results were expressed as nmol/ min mg protein (Racker, 1955).

Superoxide dismutase (SOD): was determined by measuring the inhibition of the rate of the autocatalytic adenochrome formation at 480 nm in a reaction medium containing 1 mM epinephrine and 50 mM glycine / NaOH (pH 10.20). The enzyme activity was expressed as superoxide dismutase U/ mg protein. One unit is defined as the amount of enzyme that inhibits the rate of adrenochrome formation by 50 % (Ferreira *et al*., 2013).

Protein carbonyls (PO): Protein carbonyl groups were detected with 2,4-dinitrophenylhydrazine (DNPH), which leads the formation of a stable 2,4-dinitrophenylhydrazone product (DNP). The DNP absorbs ultraviolet light so that the total carbonyl content of a protein can be quantified by a spectrophotometric assay at 370 nm (Ferreira *et al*., 2013).

Thiobarbituric acid reactive substances (TBARS): were determined in homogenates using a spectrophotometric method based on the 2-

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thiobarbituric acid reaction. TBARS was detected at 535 nm, using a spectrophotometer (Hitachi, Tokyo, Japan). Results were expressed in nmol/mg protein. (Ferreira *et al*., 2010).

Reduced glutathione concentration (GSH): was evaluated spectrophotometrically. Samples were treated with DTNB 6 mM. The absorbance was measured at 412 nm. Results were expressed as µmol/ g organ (Ferreira *et al*., 2013).

Statistical analysis: Data was expressed as mean value ± SEM (standard error of the mean). Statistical significance was calculated by the two-tailed unpaired Students t-test followed by Tukey-Kramer post-test, and a probability value of $p<0.05$ indicated a statistically significant difference.

Results

Elevated IOP is the most important risk in glaucoma damage. All animals responded with an increase in IOP after the development of the experimental glaucoma and exhibited optic nerve head damage. Table 1 shows antioxidant enzymes activities in GN and CV homogenates from all studied groups.

Table 1. Antioxidant enzymes activities in GN and VC from all studied groups.

	TRxR (nmol/min. mg protein)	GR (nmol/min. mg protein)	SOD (U/mg protein)
Geniculate nucleus			
C	14.2 ± 0.4	15.9 ± 1,5	15.0 ± 1.4
LC	15.7 ± 0.3	17.0 ± 1.9	17.6 ± 1.6
G	$7.6 \pm 0.4^{+++}$	$8.2 \pm 1.2^{++}$	$18.0 \pm 1.1^{+}$
LG	$11.6 \pm 1.5^{**}$	$14.9 \pm 1.3^{**}$	$22.1 \pm 1.9^{*}$
Visual Cortex			
C	14.9 ± 0.8	13.0 ± 1.0	4.3 ± 0.5
LC	14.8 ± 0.8	13.9 ± 0.5	6.8 ± 0.7
G	$9.9 \pm 1.0^{+++}$	$5.1 \pm 1.3^{+++}$	$6.0 \pm 0.4^{+}$
LG	$12.5 \pm 0.3^{*}$	$20.4 \pm 2.0^{***}$	$10.8 \pm 1.0^{***}$

(* *p<0.05* LG vs G,***p<0.01* LG vs G,****p<0.001* LG vs G,$^{+}$ *p<0.05* G vs C,$^{++}$*p<.0.01* G vs C, $^{+++}$*p<.0.001* G vs C)

The activities of TRxR and GR decreased 46 % (*p<0.001*) and 49 % (*p<0.01*) in GN and 34 % (*p<0.001*) and 52 % *(p<0.001*) in VC comparing G to C (Table 1). Meanwhile SOD activity showed an increase of 20 % (*p<0.05*) in GN and 40 % (*p<0.05*) in VC in G respect to C (Table 1).

LA treatment increased the activities of: TRxR 52 % (*p<0.01*) in GN, 26 % (*p<0.05*) in VC; GR 82 % (*p<0.01*) in GN and 300 % (*p<0.001*) in VC; and SOD 23 % (*p<0.05*) in GN and 80 % (*p<0.001*) in VC comparing LG to G (Table 1).

Markers of oxidative damage (PO and TBARS) are displayed in Table 2. PO increased 99 % (*p<0.001*) in GN and 138 % (*p<0.001*) in VC, meanwhile TBARS increased 34 % (*p<0.05*) in GN and 131 % (*p<0.001*) in VC in G with respect to C. LA administration diminish PO 52 % (*p<0.05*) in GN and 58 % (*p<0.001*) in VC comparing LG to G (Table 2). Damage to lipid decreased 25 % (*p<0.05*) in GN and 36 % (*p<0.05*) in VC with LA treatment (Table 2).

Table 2. Markers of oxidative damage in GN and VC homogenates in all studied groups.

	PO (nmol/mg protein)	TBARS (nmol/mg protein)
Geniculate Nucleus		
C	11.5 ± 1.5	3.6 ± 0.3
LC	11.2 ± 0.9	3.4 ± 0.2
G	$22.9 \pm 2.3^{++}$	$4.8 \pm 0.4^{+}$
LG	$10.9 \pm 1.2^{*}$	$3.6 \pm 0.3^{*}$
Visual Cortex		
C	3.9 ± 0.3	2.3 ± 0.2
CL	3.6± 0.2	2.9 ± 0.6
G	$9.3 \pm 1,3^{++}$	$5.3 \pm 0.6^{++}$
LG	$4.0 \pm 0.4^{**}$	$3.4 \pm 0.6^{*}$

(**p<0.05* LG vs G,***p<0.001* LG vs G, $^{+}$*p<0.05* G vs C,$^{++}$*p<.0.001* G vs C)

Figure 1 showed GSH levels in GN and VC in all studied groups. Levels of GSH decreased 44 % in GN (*p<0.01*) and 46 % (*p<0.001*) in CV in G compared to C (Figure 1). LA treatment raised GSH values 42 % (*p<0.05*) in GN and 73 % (*p<0.001*) in VC in LG with respect to G (Figure 1).

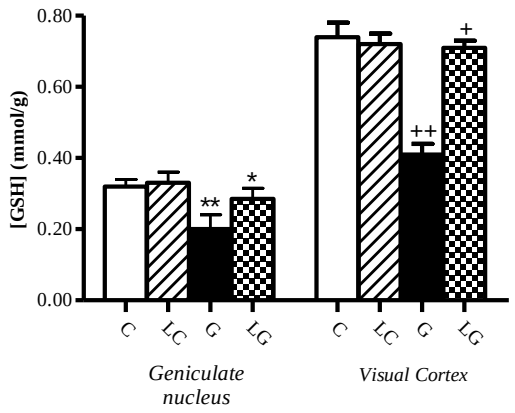

Figure 1. GSH levels in GN and VC homogenates from C, LC, G and LG. ($^{**}p<0.05$ G vs C, $^{*}p<0.01$ G vs LG, $^{+}p<0.01$ G vs C,$^{++}p<0.001$ G vs LG)

It is important to take into account that not significant differences were found between C and CL for all studied markers.

Conclusions

Both in geniculate nucleus and visual cortex of glaucoma, TRxR and GR activities are decreased whereas SOD activity is increased suggesting an adaptative response to oxidative damage. The decrease of TRxR and GR activities would lead to a deficiency in glutathione and thioredoxin recycling. The thioredoxin and glutathione systems are essential to maintain redox homeostasis in the cell so alterations in both systems could lead to an oxidative imbalance (Halliwell, 2007). Moreover taking into account that SOD catalyzes the dismutation of superoxide anion to hydrogen peroxide, the increase in SOD activity in glaucoma may lead to an increase in the concentration of hydrogen peroxide which would exacerbate this situation.

Treatment with lipoic acid on the experimental glaucoma model in rats increased the activity of TRxR, GR and SOD both in geniculate nucleus and visual cortex. Lipoic acid relief of Nrf2 repression may explain the increase in antioxidant enzymes expression (Inman *et al*, 2013)

The prooxidant markers (TBARS and PO) are increased in glaucoma suggesting an increment in active oxygen species. These results are consistent with the formation of carbonyls groups in glutamine synthase and HSp72 heat-shock protein, identified as targets of oxidative stress in an experimental glaucoma model in rats (Tezel *et al*., 2005). Unlike the products of lipid peroxidation, the carbonyls are a stable product of proteins oxidation, and can remain without being repaired or removed. Oxidized proteins not only indicate the occurrence of oxidative stress but also show the loss of functionality (Halliwell, 2007). All these findings indicate that oxidative process is exacerbated in this experimental glaucoma model and is consistent with the findings in another mouse glaucoma model that showed that in the retina lipid peroxidation and protein damage are observed (Lambert *et al*., 2008).

The administration of lipoic acid reduced the levels of PO and TBARS in LG in geniculate nucleus and visual cortex. Veskovic and collaborators (2015) showed that treatment with lipoic acid reduced the process of lipid peroxidation in a mice model with choline and methionine deficiency. These results are consistent with those found by Imnan *et al.* (2013), who in retina of DBA/2J mice showed that treatment with lipoic acid decreased levels of lipid peroxidation evaluated from the determination of malondialdehyde and 4-hidroxialkenal. It is important to note that the hydroxyl radical is the main initiator of the lipid peroxidation chain therefore a decrease in this radical level could explain the observed decrease of damage to lipids. Lipoic acid can act trapping directly this radical or can reduce its formation chelating iron, since the main mechanism by which this radical is generated in biological systems is Fenton-Haber-Weiss reaction catalyzed by this metal.

Reduced GSH levels were measured in this study as an indicator of water-soluble antioxidants concentration in the geniculate nucleus and visual cortex. Reduced GSH decreased in glaucoma probably due to the deficient recycling observed in this condition by the decrease of GR activity. Lipoic acid treatment produced a significant increase in GSH levels which could be due to its capacity of regenerating no enzymatic antioxidants by its action in the recycling of glutathione and ascorbic acid (Packer *et al*., 1997).

In summary our results showed that oxidative stress may possibly act as a risk factor in glaucoma. Lipoic acid treatment not only increased the activities of antioxidant enzymes but also reduced protein

oxidation, lipid peroxidation and increased GSH levels so it could be used as a novel therapy for reducing oxidative damage in the lateral geniculate nucleus of this glaucoma animal model. Further studies are needed to confirm beneficial effects of lipoic acid in humans and compare it with other antioxidants but it appears to be useful as it crosses the blood-brain barrier.

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