CHRONIC ETHANOL FEEDING INDUCES OXIDATIVE STRESS IN THE RAT RETINA: TREATMENT WITH THE ANTIOXIDANT EBSELEN

ESTRÉS OXIDATIVO EN LA RETINA DE LA RATA INDUCIDO POR LA ADMINISTRACIÓN CRÓNICA DE ETANOL: TRATAMIENTO CON EL ANTIOXIDANTE EBSELEN

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ABSTRACT

Objective: To assess the involvement of biochemical and functional changes to the retina after chronic ethanol intake in adult rats, and the capacity of the antioxidant ebselen to prevent these changes.

Methods: Male Sprague-Dawley rats were used in the study. They were fed an ethanol-containing liquid diet, whereas a control group was given an ethanol-free isocaloric diet. After six weeks of experiment, the eyes were extracted and homogenized without the lens, and markers of oxidative stress were assayed, i.e., glutathione (GSH) and malondialdehyde (MDA) as an intracellular antioxidant and a lipid peroxidation product, respectively. Moreover, retinal function was assessed by electroretinogram (ERG).

Results: The retinal MDA concentration was significantly increased in the ethanol-fed animals compared to controls, whereas the GSH content was

RESUMEN

Objetivo: Establecer la existencia de cambios bioquímicos y funcionales en la retina tras la administración crónica de etanol en ratas adultas, y estudiar la capacidad del antioxidante ebselen para corregir estos efectos.

Métodos: Se utilizaron ratas macho Sprague-Dawley, que fueron alimentadas con una dieta líquida con etanol, mientras el grupo control recibió una dieta isocalórica libre de etanol. Después de seis semanas, los ojos fueron extraídos y homogenizados sin cristalino, y se determinaron parámetros relevantes en la modulación del estrés oxidativo, tales como el contenido de glutatión (GSH) y de malondialdehído (MDA) como antioxidante intracelular y producto de la peroxidación de lípidos, respectivamente. Además, se comprobó la funcionalidad de la retina mediante electroretinograma (ERG).
significantly reduced in the ethanol-fed group compared to controls. Ethanol also induced a decrease in ERG b-wave amplitude. Ebselen treatment restored the MDA and GSH concentrations and ERG b-wave amplitude to control values.

**Conclusion:** These results indicate that chronic alcohol consumption alone and without the influence of nutritional factors alters the retinal redox status as well as its function (ERG). Further studies are required to better understand the protective mechanism of ebselen in this experimental model of chronic alcoholism (Arch Soc Esp Oftalmol 2007; 82: 757-762).

**Key words:** Ethanol, oxidative stress, glutathione, lipid peroxidation, electroretinogram, antioxidant, ebselen.

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**INTRODUCTION**

The chronic consumption of alcohol is highly prevalent in our society. Alcohol can exert a toxic action due to its direct effect on the generation of free radicals or through the metabolites thereof (1), mainly acetaldehyde (2). Oxidative stress is defined as an imbalance between oxidative and anti-oxidative agents in which the oxidative agents are the majority (3). It has been proved that the chronic intake of ethanol leads to an increase of lipid peroxidation products and a reduction of antioxidative factors (4) such as glutathione (GSH) and its related enzymes (5). Free radicals are able to produce damages in different tissues, and at present researchers are investigating the possibility of utilizing antioxidants as neuroprotectors in tissues damaged by ethanol (6).

The retina is the neurosensory tissue of the eye and its membranes are extremely rich in polyunsaturated lipids. This characteristic makes it particularly sensitive to free oxygenated radicals and to lipids peroxidation (7). Several eye diseases are related to oxidative stress, including diabetic retinopathy and uveitis (8,9). Data published by our lab describe a reduction in the content of GSH as well as a higher concentration of malondialdehyde (MDA) in the optic nerve of alcoholic rats (10). These results, together with the importance of oxidative stress in retinal diseases and chronic alcoholism, gave us reason to investigate the role of these parameters in the retina and to essay a treatment with the antioxidant ebselen.

Ebselen is a biologically active selenium-organic compound with anti-inflammatory properties (11). Its activity is similar to that of glutathione peroxidase and its effect has been documented as a capturing agent of peroxynitrites (12). At present the role of this antioxidant is being researched in diseases such as pigmentary retinosis, diabetic retinopathy and uveitis.

**SUBJECTS, MATERIAL AND METHODS**

For this study 24 male Sprague-Dawley rats were utilized (6 in each group). The manipulation and care of the rats was carried out in accordance with the international regulations of the EEC, (order 86/609/CEE) and the A.R.V.O. (Association for Research in Vision and Ophthalmology).

The rats, weighing 300-325 g, were divided in groups of twelve. These two groups were paired according to weight for controlling their food intake. The first group (ethanol) received the Lieber-De Carli diet (13), which is usually utilized for chronic alcoholism models. The ethanol group was in turn...
divided in groups of six rats each, administering ebselen to the rats of one group together with the alcoholic diet (0.1 mg/ml of diet). The alcohol values ranged between 195-225 mg/dL during the last five weeks (blood and analysis obtained from the tail of randomly selected rats during this period, at a rate of two animals per week). The control group was given an isocaloric mixture of dextrinated maltose instead of ethanol and was also divided into groups of six. One of these groups was given ebselen together with the diet.

The animals were administered said diet for six weeks and kept in individual cages in the Department of Physiology, Pharmacology and Toxicology of the Cardenal Herrera-CEU University under controlled temperature (20º) and humidity (60%) and constant cycles of light and darkness of twelve hours each. Immediately after being sacrificed, both eyes were enucleated and the lens extracted. The samples were homogenized in potassium phosphate buffer tampon 0.2 M, pH 7. It has been demonstrated that the content of antioxidants in these samples corresponds to the retina in 97% (14).

In order to quantify the MDA, we determined the level of the complex formed between MDA and thiobarbituric acid, following a modification of Richard’s method (15) described by our group (16), utilizing a liquid high resolution chromatography device (HPLC, Waters). The GSH concentration was determined with a modification of the procedure described by Reed et al. (17), based in the reaction of iodineacetic acid with the thiol groups followed by a chromophore derivatization of the amino groups with Sanger reactant (1-fluoro-2,4-dinitrobenzene), giving rise to derivatives which were quickly separated by means of HPLC, thus allowing a quantification of nanomolar concentrations of GSH. The proteins content was determined according to the method proposed by Lowry (18). The statistical analysis was carried out according to the t for student test, with p < 0.05 being considered significant. The data are expressed as mean and standard deviation (SD).

The ERG was measured with the rats previously anesthetized with ketamine (100 mg/kg weight) and azepromazine (2.5 mg/kg weight), and adapted to darkness. Anesthetic and miotic eye drops were administered. A golden conjunctival contact loop electrode was utilized together with a reference electrode in the back of the head and a mass electrode on the tail of the rats. The stimuli were flashes with a maximum duration of 4 ms [average 2; range 100; intensity 1 (0.06 x 22 lumen sec/ft²)]. A white standard flash was placed at the front with a filter having 2.5 logarithmic units of optical density. The passing range of the amplifier and preamplifier were established at 3-50 Hz. The records were kept in a MacLab computer (Castle Hill, Australia). The a and b amplitude of ERG waves were measured.

RESULTS

The mean volume of diet ingested by the various groups are shown in table one. No significant differences were found in the evolution of the mean diet volume consumed by each group.

Figure one shows the MDA values in the retina. In the ethanol group we found MDA values higher than in the control group (ethanol 1.42 SD 0.17, control 0.70 SD 0.04 nmol/mg protein) whereas the administration of ebselen reduced said values, making them equal to the control values (ethanol+ebselen 0.76 SD 0.16, control+ebselen 0.78 SD 0.08 nmol/mg protein).

The GSH values in the samples obtained are shown in figure 2. The GSH concentration went down in the retina of alcoholic animals (ethanol, 759

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<th>Table I. Mean diet volume ingested by the groups</th>
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<td>Diet volume ml/dat (mean ± SD)</td>
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<td>Control</td>
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Fig. 1: MDA concentrations (nmol/mg protein) in retina of the various groups after six weeks of the experiment. * p<0.05 vis-à-vis all the other groups.
12.18 SD 2.37, control 23.38 SD 1.69 nmol/mg protein). Treating alcoholic animals with ebselen caused an increase of GSH values, making them equal to the control values (ethanol+ebselen 19.26 SD 2.88, control+ebselen 21.06 SD 3.45 nmol/mg protein).

Figure 3 shows the values of the ERG b wave width, which is significantly reduced in alcoholic animals after six weeks of alcoholic diet (ethanol, 126.0 SD 8.9, control 163.7 SD 12.7 µV). Treatment with ebselen restored that reduction in the width of the b wave (ethanol+ebselen 150.0 SD 9.0, control+ebselen 191.7 SD 12.8 µV). No significant differences were found in the width of a wave amongst all the groups of the study (data not shown).

**DISCUSSION**

For many years, the existence of a genuine alcoholic optical neuropathy was denied together with the rejection of a toxic effect of ethanol. The visual loss which takes place in many alcoholics has generally been assigned to a condition known as tobacco-alcoholic amblyopia, attributing a synergistic effect between alcohol and nicotine, together with vitamin deficiencies secondary to a poor diet, all of which leads to the loss of central vision (19). In addition, it has recently been described that tobacco-alcoholic amblyopia causes damages in the retina in addition to the optic nerve (20).

In experimental models with chronic administration of ethanol, researchers have recently demonstrated an increase in the concentration of lipids peroxidation products in the optic nerve (9). The fact that the membranes of the external segments of the rods contain a very high concentration of long chain poly-unsaturated fatty acids (21) makes them particularly vulnerable to oxidative damage.

The results obtained in this study with homogenized eye without lens of chronic alcoholic rats, in which the only difference with the control group is the alcohol supplied in the diet, shows an increase of products derived from lipid peroxidation (MDA), as well as a reduction in the level of endogenous antioxidants (GSH). This evidence supports the role of oxidative stress in eye pathologies associated to alcohol. The literature provides many examples which prove that 97% of metabolites of the homogenized eye without lens corresponds to the retina (14). The high concentrations of MDA in the eyes of alcoholic rats confirmed the importance of lipid peroxidation in the damages caused by the chronic intake of ethanol. There is a general consensus about the determination of MDA with HPLC effective markers of the involvement of oxidative stress in a pathological condition and their usefulness to assess the effect of antioxidant treatment (22). The data provided in this paper demonstrate a normalization of the levels of MDA and GSH in alcoholic rats when administering ebselen together with the diet, thus suggesting a possible treatment to avoid damage caused by oxidative stress as a result of the chronic intake of ethanol.

At the electroretinographic level, the ethanol group had a b wave mean width of 120.0 SD 8.9 µV, in practical terms a reduction of 25% with respect to the control value. This important reduction in wave amplitude matches another study which proved that the ERG changes in patients with tobacco-alcoholic amblyopia generally originated in the internal ganglion and nuclear cell layer of the retina (20). Our data suggest that, in addition to the optic nerve, the
retina is also affected by the chronic consumption of ethanol. An ERG allows to establish a relationship between the oxidative stress parameters and the functional integrity of the retina. The results shown imply the existence of a pathology in the eye, specifically in the retina, attributable to the chronic intake of ethanol as the only etiological level.

The administration of ebselen to alcoholic rats produced a recovery up to the control values in the width of b waves in addition to a normalization of the oxidative stress and relatives. The use of ebselen, an agent with an activity similar to that of glutathione peroxidase and which captures peroxinitrites (3) could be considered as an adequate method for the treatment of the alterations caused by ethanol and observed in this study. Considering the penetration capacity of ebselen through the blood-retina barrier (23), its low toxicity and the fact that our group has previously demonstrated its efficiency in the treatment of other eye pathologies (9,14) as well as for the neurotoxicity induced by ethanol (6), the results shown above allow ebselen to be proposed as a possible treatment for alcoholic patients suffering eye problems. Even so, additional work is required to confirm the action of ebselen in this chronic alcoholism model.

REFERENCES


