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The Role of Vitamin E in Retina Due To Copper Toxicity

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Abstract

Copper at a higher level becomes toxic and it can catalyze the formation of highly reactive hydroxyl radical. The aim of the present study is to determine functional and molecular alterations of rat retina caused by copper toxicity and the role of vitamin E in protection. Forty albino rats (3 moonths) weighing 200-225 g were divided into 4 groups. Group I as control group received 0.5 mL normal saline.; Groups II and III received 0.5 and 1.5 mg/kg copper chloride ($CuCl_2.2H_2O$) as intraperitoneally injection in single dose daily for 21 days and group IV was animals received 1.5 mg/kg copper chloride in combination with intraperitoneally injected with vitamin E at a dose of 50 IU/kg body weight. Electrretinogram results indicate significant decrease ($p<0.05$) appeared in a,b value and their implicit time of treated animals with 1.5mg copper. Also a time-dependent appearance of the typical ladder pattern of internucleosomal fragmentation, a characteristic of apoptosis was found in the same dose and an improvement in treated groups with vitamin E. Copper induced function loss and apoptosis to retinal tissue via generating free radical especially in higher doses. Vitamin E may strengthen the antioxidant defense system by inhibiting protein oxidation and enhancing the activity of antioxidant enzymes.

Keywords: retina, copper, apoptosis, ERG, DNA, toxicity

Introduction

Copper (Cu) is one of many metal ions that are required for essential body functions. Cu at a higher level becomes toxic and it can catalyze the formation of highly reactive hydroxyl radical. Copper is present throughout the brain and is most prominent in the basal ganglia, hippocampus, cerebellum, numerous synaptic membranes, and in the cell bodies of cortical pyramidal and cerebellar granular neurons [1]. Enzymes in the central nervous system that depend on Cu for their function include tyrosinase, peptidylglycine α-amidating mono-oxygenase, copper/zinc superoxide dismutase, ceruloplasmin, hephaestin, dopamine-β-hydoxylase, and cytochrome ***c*** oxidase [2-4]. Excessive Cu intake can occur via the consumption of Cu-rich foods such as liver, seafood, nuts, whole grains, and dried fruits. Cu exposure can also occur due to exposure to residues of pesticides [5] commonly used in agriculture as well as via drinking water contaminated by environmental pollution [6] or by corrosion of Cu water pipes [7]. Ingested Cu can participate in reactions that most diets contain enough Cu (1-5 mg) to prevent a deficiency and not enough to cause toxicity. The United States Environmental Protection Agency (2013) [8] has set the maximum contaminant level goals for Cu at 1.3 mg/L or 1.3 ppm. Cu is implicated

directly or indirectly in the pathogenesis of numerous neurological diseases, including aceruloplasminemia, Alzheimer disease, amyotrophic lateral sclerosis, Huntington disease, Menkes disease, occipital horn syndrome, Parkinson disease, prion disease, and Wilson disease (WD). Gahlot and Rathakar 1981 [9] reported that chronic copper toxicity was produced in pigmented rabbits by injecting Copper Sulphate intraperitoneally. Electroretinography (ERG) repeated after 4 weeks showed an extinguished response. Histologically retina showed degeneration of photo receptor cells and migration of pigment.

Excessive Cu also may deposit in the eye, causing, a pathognomonic sign, the Kayser-Fleischer (KF) ring, is an annular deposition of copper in the periphery of the cornea which is present in about 95% of patients with neurological symptoms of copper toxicity[10]. Cu can also accumulate in the lens and cause "sunflower" cataracts. About ≈95% of patients with a neurological presentation manifest the Kayser-Fleischer ring compared with ≈65% of those with a hepatic presentation [11].

Albrecht et al., 2012[12] analyzes the retinal changes in WD patients. Morphological changes measured by OCT device with Delayed visual evoked potentials (VEPs) as functional parameters and correlated these findings with laboratory parameters and a clinical WD score suggest changes to the visual system and potential structural changes of the retina.

The retinal degenerations in WD and Menkes diseases could result from abnormal systemic Cu levels or loss of retinal copper transporters. ERGs are abnormal in patients with WD; Patients with WD have reduced amplitude of photopic a-waves [13].

Gahlot 1979 [14]reported that treatment of retinitis pigmentosa patients with a decoppering agent and low Cu diet suggested that both acuity and fields of vision improved after a few months of treatment. Intracellular copper deposits impede inhibitor of apoptosis proteins (IAPs), which eventually causes apoptotic cell death [15]

It is clear that from earlier study by Temiz et al., 2018 [16] Vitamin E and melatonin are protective against liver damage caused by Cu. Tocopherol, the most effective and most powerful form of vitamin E, is an important antioxidant compound with many physiologic functions, including maintenance of plasma membrane integrity, cell signaling and cell cycle regulation, cell adhesion, platelet aggregation, smooth muscle cell proliferation, and immune function [17].

The objective of the present study is to determine functional and molecular alterations of rat retina caused by Cu toxicity and role of vitamin E in protection of Cu toxicity.

Materials and Methods

Rats were randomly selected from the animal house facility at the Research Institute of Ophthalmology, Giza, Egypt. The experimental protocol was approved by the local ethical committee that applies ARVO statements of using animals in ophthalmic and vision research. Rats were kept in rat cages in well ventilated house, temperature of 27°C -30°C, 12h natural light and 12h darkness, with free access to tap water and dry rat pellet. All chemicals were purchased from Sigma Chemical Co. (St.Louis, MO, USA). A pilot study was done to Wistar age matched-albino rats (3 moonths) weighing 200-225 g comprised of both sex by measuring all parameters before treatment and we found no significant difference. Hence we randomly selected 40 rats and divided them into 4 groups. Group I as control group received 0.5 mL normal saline.; Groups II and III received 0.5 and 1.5 mg/kg copper chloride ($CuCl_2.2H_2O$) as intraperitoneally injection in single dose daily for 21 days and group IV was animals received 1.5 mg/kg copper chloride in

combination with intraperitoneally injected with vitamin E at a dose of 50 IU/kg body weight.

Electroretinogram

The protocol used for ERG acquisition was the one suggested by the International Society for Clinical Electrophysiology of Vision (ISCEV) modified for acquirement of some additional information in experimental studies. The ISCEV protocol and the recommendation for each laboratory to determine its own normative values are extremely important for a high quality test. Normative values from different laboratories may differ for several reasons including different equipment, different electrodes, and different settings on the Ganzfeld. For obtaining scotopic answers, animals were adapted in the dark for 30 minutes They were anesthetized intraperitoneally ketamine and xylazine (100 and 5 mg/kg respectively), and after establishing the anesthesia, animals were placed on the pad of an operating table where their body temperature was maintained at 37°C. Each rat was positioned with its head resting to one side and local anesthetizing eye drops were also applied. . The pupil of the recorded eye was dilated with topical 1% mydriacy and was submitted to stimuli. A white flash was used in this work with fixed intensity 4 lux and duration 0.2 seconds. . ERG was recorded by using sensor PS-2111 and its electrodes (PASCO, Roseville, CA) which connect to PASPORT interface direct to the computer. One electrode was placed at the corneal periphery as active electrode. The other two electrodes were placed on the skin of the lower eyelid and on the ear, as reference and earthed electrodes. The electrodes were placed on the skin after removal the hair. The resulted electrophysiological signals were collected and analyzed by data studio 1.9.8 software (PASCO, Roseville, CA). A and b waves were recorded and their amplitude and implicit time were analyzed. A wave amplitude was measured from baseline to minimum amplitude registered after presentation of stimuli. Implicit time was measured from the beginning of luminous stimulus until the a wave peak. B wave amplitude was measured from a wave peak to b wave peak, and the implicit time of b wave corresponded to the necessary time for that peak.

DNA Fragmentation Studies by Gel Electrophoresis

Retinal DNA analysis was performed using a previously described procedure by **Lam et al., 1999** [18]. Briefly, after enucleation, the retinas were dissected from the retinal pigment epithelium and choroid, frozen in liquid nitrogen, and stored at –80°C. Retinal DNA was extracted using the standard phenol/chloroform/ isoamyl alcohol (25:24:1) method. The concentrations of DNA in each sample were determined by measuring the absorbance at 260 nm. Samples of 10 mg of DNA were loaded in each well of a 2.0% agarose gel. Electrophoresis was performed at 3 to 5 V/cm. DNA was stained with a 1:10,000 dilution of SYBR Green I (Molecular Probes, Eugene, OR) in Tris borate EDTA (TBE) buffer (89 mM Tris base, 89 mM boric acid, 1 mM EDTA, pH 8), visualized by transillumination with UV light, and photographed.

Statistical Analysis

Data were represented as the mean±SD. For comparison between multiple groups the analysis of variance (ANOVA) procedure was used, where a commercially available software package (SPSS-11, for windows) was used and the significance level was set at $P<0.05$

Results

Table (1) illustrated results of ERG that indicating a-wave and b-wave parameters which are the amplitude, the implicit time and b/a ratio for rats injected with Cu with two doses (0.5, 1.5 mg Cu) and rats treated with vitamin E compared to control. The amplitude of a and b waves were 74.19±16µv and 154.39±20µv, respectively. Their implicit times were 39.98±4 msec and 44.98±3 msec, respectively.

The results indicate significant differences decrease ($p<0.05$) appeared in a,b value and their implicit time of treated animals with 1.5mg Cu. Also, there were no significant differences appeared for ERG parameters of injected animals with 0.5 mg Cu or for animals group treated with vitamin E after injection of 1.5 mg Cu.

Table 1 : ERG parameters to animals injected with copper and those treated with vitamin E compared to control

	a-wave		b-wave	
	Amplitude (µv)	**Implicit time (msec)**	**Amplitude (µv)**	**Implicit time (msec)**
Control	74.19±16	39.98±4	154.39±20	44.98±3
0.5 mg Cu	49.61±23	30.25±6	114.13±22	36.53±4
1.5 mg Cu	24.27±10*	26.50±3*	57.42±17*	29.46±3*
1.5mgCu+ Vit E	66.78±18	35.04±5	125.91±16	40.11±3

*Statistical significant difference ($p<0.05$)

Electrophoresis by agarose gel of retinal DNA results from all groups compared to control appeared in figure (1). Apoptosis characterization as typical ladder pattern fragmentation was appeared and no ladder was found in the control or in group treated with 0.5 mg Cu. Ladder pattern was noticed in retinal tissue of injected animals with 1.5 mg of Cu. But after treated with vitamin E, a faint ladder pattern was observed. Table (2) showed the numbers of bands, base pair (bp) and the percentage of each bp for ladder and all the studied groups. The numbers of ladders were found to be 10, 1,1,3,2 bp for marker, control, injected animals with 0.5 mg Cu, injected animals with 1.5 mg Cu and those treated with vitamin E, respectively.

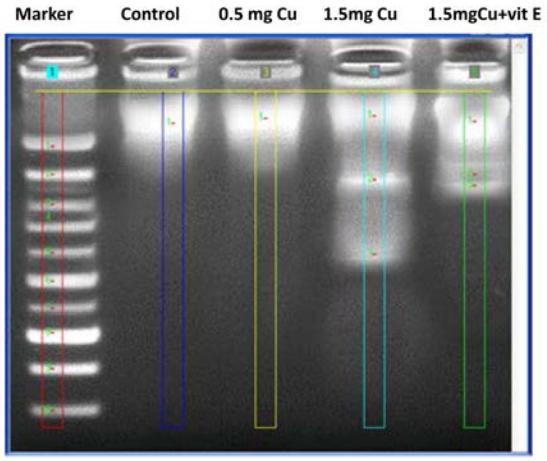

Figure 1: DNA fragmentation for retina to all the studied rats groups

Table 2: Bands and base pair percentage for all groups studied and the marker

Lanes:	Ladder		Control		0.5 mg Cu		1.5 mg Cu		1.5mgCu+ Vit E	
Bands	**Base Pair**	**%**	**bP**	**%**	**bP**	**%**	**bP**	**%**	**bP**	**%**
1	1000	16.03	1089	100	1108	100	1118	32.835	1095	62.67
2	900	11.06					883	25.352	900	37.33
3	800	6.78					593	41.776		
4	700	7.03								
5	600	5.78								
6	500	13.45								
7	400	5.47								
8	300	16.48								
9	200	10.90								
10	100	6.97								
Sum		100		100		100		100		100
In Lane		100		100		100		100		100

Discussion

Cu is an essential element, dispersed extensively throughout different animal tissues. It participates in the formation of a number of metalloenzymes, including catalase, cytochrome oxidase, and peroxidase [19]. Cu is a catalyst for oxidative stress via the creation of ROS and peroxidative damage of membrane lipids. Cu ingested from the gastrointestinal tract enters into systemic circulation and binds to plasma amino acids and albumin, from where it is transported to the liver and involved into ceruloplasmin formation [20]. Retinal Cu deposition should affect the conduction properties of the retina, which is an

extension of the central nervous system [21]. Another study confirmed that OCT, ERG, and PVEP are potentially valuable clinical tools for the investigation of organ injury in WD and provides a foundation for investigating further correlations between retinal pathology and clinical characteristics of WD patients, as well as patient response to anti-Cu therapy [13]. In addition to the free radical-induced oxidative damage, information available suggests that the cellular response to Cu overload, particularly at the early stages of Cu accumulation, involves more specific mechanisms and pathways. This include regulation of lipid metabolism, antimicrobial defense, neuronal activity, resistance of tumor cells to chemotherapeutic drugs, kinase-mediated signal transduction, and other essential cellular processes [22]. While the mechanism of these actions remains to be established, many regulatory and signaling events are associated with changes in the intracellular localization and abundance of Cu transporters, as well as distinct compartmentalization of Cu itself. Several other possible mechanisms of Cu toxicity are listed below: Intracellular copper deposits impede inhibitor of apoptosis proteins (IAPs), which eventually causes apoptotic cell death [12]. We believe that the prolonged latencies are likely to reflect a slowed conduction velocity of the visual tract caused by Cu deposits. High levels of Cu in cells could also lead to precipitation of cupric salt crystals (as occurs in WD) which could damage cell organelles [23]. The results indicated that excess Cu affects function of the retina accompanied by apoptosis and the improvements that appeared after vitamin E. The antioxidant effect of vitamin E is mediated by oxidation of the chromanol ring. Phenolic hydrogen is donated to a fatty acyl free radical to protect the polyunsaturated fatty acids from attack [24]. On the basis of this assumption, it is thought that vitamin E augments the activity of antioxidant enzymes by minimizing oxidative stress. It is likely that the effects of vitamin E observed in the present study are mediation by protection of the microsomal membrane against peroxidative compounds such as oxygen-metal complexes and chain-breaking antioxidant activity. In this experiment, regardless of the dose of copper administered to rats for 4 weeks, Ingested Cu can participate in reactions that result in the production of free radicals such as superoxide anion (O2 •-). The transformation of O2•- into hydroxyl radical (OH•), which has a higher reaction capacity in the Haber-Weiss and Fenton reactions, is the primary factor associated with oxidative stress resulting in tissue damage. Vitamin E may strengthen the antioxidant defense system by inhibiting protein oxidation and enhancing the activity of antioxidant enzymes.

The loss of ceruloplasmin synthesized in the liver may have influenced iron accumulation. Ceruloplasmin contains more than 95% of the Cu in human plasma. It is synthesized mainly in the liver. Ceruloplasmin can oxidize ferrous iron to ferric iron. In some experiments, ceruloplasmin was shown to play a role in the mobilization and oxidation of iron from tissue stores with subsequent incorporation of ferric iron into transferrin [25]. aceruloplasminemia characterized by mutations in the ceruloplasmin gene and iron accumulation in the basal ganglia as well as in parenchymal tissues in the central nervous system is caused by a total deficiency of ceruloplasmin ferroxidase activity [26]. We speculate that the free radical reaction induced by iron may provoke such a nuclear change. Ferrous iron is the electron donor in the presence of hydrogen peroxide and generates a hydroxyl radical. Thus the retinal changes function may be due to secondary iron accumulation induced by Cu excess. Although free radicals tend to impair first the photoreceptor outer segment which is rich in unsaturated fatty acids, the inner nuclear layer receives the greater

damage and this may be explain copper toxicity to the retina.

Conclusion

Cu induced function loss and apoptosis to retinal tissue via generating free radical especially in higher doses. Vitamin E may strengthen the antioxidant defense system by inhibiting protein oxidation and enhancing the activity of antioxidant enzymes.

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