The Preventive Effects of Adrenalectomy in Methanol Intoxication on Photoreceptors in Rabbit Retina (*Oryctolagus cuniculus*): Ultrastructural Study

A. Esfandiari¹ and A. Aliabadi²

¹Department of Basic Sciences of Veterinary Medicine, Kazerun Branch, Islamic Azad University, Kazerun, Iran.
²Department of Surgical Sciences of Veterinary Medicine, Kazerun Branch, Islamic Azad University, Kazerun, Iran.

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Abstract

In this work the protective effect of adrenalectomy against the methanol intoxication in photoreceptor layer of rabbit retina was studied. Fifteen female White New Zealand rabbits were used in this study, and were divided into three groups: Control group, Experimental group I (received 4 gram/kilogram methanol), Experimental group II (adrenalectomized and received 4 gram/kilogram methanol). Then after, the retina was separated and observed by transmission electron microscope.

The major sign of pathology such as outer segment loss, disorganized inner segment and condensed and pyknotic nuclei in outer nuclear layer of photoreceptor layer were seen in experimental group I. But normal photoreceptor layer were obvious in adrenalectomized animals in experimental group II.

These observations showed that the adrenalectomy could improve the damage of the photoreceptor layer of the retina against the oxidative stress of methanol intoxication.

Keywords: Photoreceptor layer, Methanol, Adrenalectomy, Rabbit

Introduction

The retina is an important tissue for sight and light-sensitive tissue lining the inner layer of the eyeball. Methanol intoxication causes damage and toxic injury to the retina (Eells et al., 2003). Seme et al. (2001) indicated that methanol intoxication inhibits retinal mitochondrial function and increases oxidative stress. The severity of oxidative stress induced retinal damage is closely related to the hormonal state (Lopez et al., 2008). Glucocorticoids cause oxidative processes by decline in antioxidant defenses (Zafir and Banu, 2009). Also, super oxide dismutase, catalase and glutathione reductase decreases in the activities by actions of corticosterone (Zafir and Banu, 2009). In addition, glucocorticoids can cause—neurotoxicity...
through different mechanisms (Madrigal et al., 2006). As well as, the stress response mediated by the adrenal gland involved in the initiation of this neuroprotective phenomenon and adrenalectomy itself yielded retinal protection (Tanito and Anderson, 2006; Lopez et al., 2008). The association of experimental conditions that stress related adrenal gland function and increase susceptibility of the retina to methanol intoxication induced damage and hormonal function led to this research. The present study has been designed to investigate the retinal damage of methanol intoxication after bilateral adrenalectomy.

Materials and Methods
Fifteen female White New Zealand rabbits aged 6 months were kept in a cyclic light environment (12 hours light: 12 hours darkness) and temperature (23-25 degrees Celsius) for 2 weeks. Bilateral adrenalectomy was accomplished by the dorsal approach. All procedures and care of the animals were conducted following protocols approved by the local ethical committee and all studies were performed in accordance with in the Guide to the CALAM Standards of Veterinary Care (Patricia, 2008). Three groups of animals, namely control group (N=5), experimental group I (received 4 gram/kilogram methanol by intraperitoneal injection for 30 days) and experimental group II (received 4 gram/kilogram methanol by intraperitoneal injection for 30 days prior to bilateral adrenalectomy). The retinal sample separated near the optic disc similar to in our previous study (Esfandiari et al., 2009; 2012; 2013; Goodarzi et al., 2014). The cornea, lens and vitreous body of eye were removed and placed in 4% glutaraldehyde for 4hr. Then, the specimens transferred to 1% osmium tetroxide and dehydrated through a graded ethanol series. The retinas were embedded in resin. Semi thin and ultrathin sections of the retina were stained with toluidine blue and lead citrate and uranyl acetate, respectively. The retina processed for transmission electron microscope (Philips CM-10, Eindhoven, Netherlands).

Statistical analysis
The SPSS version 16 was used for statistical analysis and analyzed with one-way ANOVA and post hoc Tukey test. The significance level was set at p≤0.05.

Results and Discussion
The ultra structural study of the photoreceptor layer in control group showed that the outer segments exhibited bi-membranous discs. The organized inner segment consists of mitochondria near the outer segment. The outer limiting membrane included zonula adherents' junction. The outer nuclear layer contained nuclei of rod and cone cells with normal heterochromatin (Fig 1). Histopathological changes were appeared in the photoreceptor layer in the experimental group I, with evidence of outer segment loss, disorganized inner segment, mitochondria loss and pyknotic and condensed nuclei (Fig 2). Significant
destruction of photoreceptor layer, as determined by morphometry of the photoreceptor layer thickness, occurred in experimental group I. On the other hand, the photoreceptor layer of adrenalectomized rabbits was distinctly different from the experimental group I. There was demonstrable improvement in adrenalectomized responses. The normal outer segment, organized inner segment and normal outer limiting membrane were seen but some pyknotic nuclei were obvious in the experimental group II (Fig 3). The thickness (means) of the photoreceptor layer in the control group was 91.76±1.84 microns, and that of the experimental groups I and II were 71.86±4.72 and 89.90±1.35 microns respectively.

The present research showed that adrenalectomy induces the photoreceptor damage in oxidative stress of methanol intoxication. The methanol intoxication resulted in severe damage to the photoreceptor layer and a resulting reduction in the thickness of the photoreceptor layer, as previously reported (Seme et al., 2006; Alla El-Din et al., 2011; Esfandiari et al., 2012; Zarenehzad et al., 2013;). The adrenal hormone influences the damaging effect of oxidative stress on photoreceptor cells of retina (Julian et al., 2005; Lopez et al., 2008; Carter et al., 2009). However, the photoreceptor pathology was much more severe in the oxidative stressed of methanol intoxication animals (Paula et al., 2003; Williams, 2008) than in adrenalectomized rabbits. Lopez et al. (2008) concluded that the oxidative stress of continuous illumination causes an increase of glucocorticoids level and induced retinal damage but adrenalectomy will improve the damage of retina. The removal of the adrenal gland improved the damaging effects of methanol on photoreceptor layers in our research. Results of Lopez et al. are consistent with the results of our experiment. Glucocorticoid-dependent death or survival is directly associated with the selective expression of the proapoptotic and antiapoptotic molecule (Cubilla et al., 2013). Therefore, we used the ratio proapoptotic molecule to the antiapoptotic molecule to evaluate the role of glucocorticoids under our experimental condition as described by Cubilla et al., (2013). So this ratio decreased in our experimental conditions in photoreceptor layer. On the other hand, Lin et al. (2004) concluded that corticosterone administration caused oxidative stress in broiler chicken. Also, glucocorticoids enhance oxidative stress induced neuronal degeneration (Lopez et al., 2008). So the adrenalectomy can probably be reduced by damage. As well as, reducing glucocorticoids by adrenalectomy increases the secretion of the corticotrophin-releasing hormone itself is damaged (Pedersen et al., 2001; Zafir and Banu, 2009). In addition, the measurements of photoreceptor layer decreased in experimental group I with significant difference compared with control group (p≤0.05) and increased in experimental group II with significant difference compared with experimental group I (p≤0.05). The morphomet-

rical results of this study that corroborates the electron microscopical study in this experiment resembles the results described by Tanito and Anderson (2006) and Lopez et al., (2008).

**Conclusion:**
The results of this study indicate that the bilateral adrenalectomy is useful in functional recovery of the photoreceptor damage after methanol intoxication and oxidative stress, these evidences further support the idea that adrenal hormones could facilitate methanol-induced photoreceptor degeneration.

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**References**


Correspondence: Arash Esfandiari, Department of Basic Sciences of Veterinary Medicine, Kazerun Branch, Islamic Azad University, P.O. Box 73135-168, Kazerun, Iran. Esfandiari.arash@gmail.com
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Fig (2): Electron micrograph of the photoreceptor layer in experimental group I. The outer segment (OS), the outer segment loss (asterisk), disorganized inner segment (IS), the outer limiting membrane (arrowhead), the outer nuclear layer (ONL), condensed nuclei (arrows) and pyknotic nuclei (wavy arrows). (Staining with lead citrate and uranyl acetate) (×2950)

Fig (1): Electron micrograph of the photoreceptor layer in control group. The outer segment (OS), the outer segment of rod cell (R) and cone cell (C), the inner segment (IS), the outer limiting membrane (arrowhead) and the outer nuclear layer (ONL). (Staining with lead citrate and uranyl acetate) (×2950)
**Fig (2):** Electro-micrograph of the photoreceptor layer in experimental group I. The outer segment (OS), the outer segment loss (asterisk), disorganized inner segment (IS), the outer limiting membrane (arrowhead), the outer nuclear layer (ONL), condense nuclei (arrows) and pyknotic nuclei (wave arrows). (Staining with lead citrate and uranyl acetate) (×2950).
**Fig (3):** Electro-micrograph of the photoreceptor layer in experimental group II. The normal outer segment (OS), organized inner segment (IS), the mitochondria (thick arrow), the outer limiting membrane (arrowhead), the outer nuclear layer (ONL) and pyknotic nuclei (wave arrows). (Staining with lead citrate and uranyl acetate) (×2950)