The protective effects of erythropoietin on photoreceptor damage by formaldehyde

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Abstract

Introduction: The photoreceptor layer of retina has important role for sight. The previous study showed that accidental formaldehyde injection caused ischemia and damage in the retina. On the other hand the erythropoietin prevents neuronal injury of ischemic damage. Objectives: The aim of this study was to survey the effect of erythropoietin on retro bulbar formaldehyde injected photoreceptor layer of rat retina.

Materials and methods: 30 adult rats were used and divided into three groups: 1- control group, 2- formaldehyde group (1 ml retro bulbar injected by 10% formaldehyde solution), 3- erythropoietin group (5000 units/kg immediately intraperitoneally injected by erythropoietin after formaldehyde injection for 7 days). The photoreceptor layer of retina studied using a transmission electron microscope. Results: Our observation showed that disorganization and vacuolization in outer segment and inner segment, pyknotic and karyolysis in outer nuclear layer were seen in formaldehyde group. But the minor sign of pathology such as lightly vacuolization in inner segment were obvious in erythropoietin group. Conclusion: We concluded that formaldehyde caused damage in photoreceptor layer and erythropoietin was improvement this injury.

Keywords: Erythropoietin, Formaldehyde, Photoreceptor layer, Rat

Introduction

The retina is a light-sensitive layer of tissue, lining the inner surface of the eye. The retina consists of several layers of neurons interconnected by synapses. The only layer that is directly sensitive to light is the photoreceptor layer. The photoreceptor layer has an important role in sight and contains rod and cone cells. Formaldehyde is commonly used as a fixative in medical laboratories. Ocular exposure to formaldehyde produces irritation and
lacrimation. Depending on the concentration, formaldehyde solutions may cause irritation or corneal opacification and loss of vision (Witek et al, 1987). Accidental formaldehyde injection is paranormal and occurs when formaldehyde is confused with lidocaine in surgery room (Soltan & Hashemi, 2004). Formaldehyde causes ischemia (Soltansanjari & hashemi, 2004). Previous research indicated that the formaldehyde has toxic effects such as: oxidative stress in tissue reacts with glutathione and formate, chromosome damage and cellular apoptosis (Matsuka et al, 2010; Just et al, 2011; Anderson et al, 2010; Tang et al, 2011). On the other hand, erythropoietin stimulates the bone marrow to produce more red blood cells. The resulting rise in red blood cells increases the oxygen-carrying capacity of the blood (Koury et al, 2002). Also, erythropoietin decreases neuronal injury caused by ischemic damage (Zhang et al, 2008; Mc Vicar et al, 2011). Recent studies showed the physiological role of erythropoietin with central nervous system. There is an activation of erythropoietin of many intracellular pathways such as mitogen-activated protein kinase (MAPK), which is associated with cell survival and inhibits the apoptosis of erythroid cells (Jelkmann & Wagner, 2004; Ratajczak et al, 2001). Additionally, erythropoietin caused proliferation and maturation of erythroid precursor cells (Fisher, 2003). Also, erythropoietin produced hematopoietic cytokine by the kidney in hypoxia. So, treatment with erythropoietin protects cultured neurons from hypoxia and therapeutic strategies retinal or central nervous system regions ischemic injury (Lewczuk, 2000). Thus, in this research we evaluated neuroprotective effect of erythropoietin on ischemic damage of formaldehyde injection by transmission electron microscope.

**Materials and methods**

Experimental design Thirty male Wistar rats were maintained on a 12h light/12dark light cycle and temperature (22-24°C). Rats aged four months and 250-300gr body weight were used. All experiments conformed to the CALAM standards of veterinary care (Patricia, 2008). The animals were divided into three groups: 1-control group, 2-formaldehyde group (0.2 ml retro bulbar injection of 10% formaldehyde solution for one dose in one eye) 3-erythropoietin group (erythropoietin at 5000 units/kg was injected intraperitoneally immediately after formaldehyde injection for 7 days) (Schwartzenberg et al, 2006). The animals were anesthetized with an intraperitoneal injection of ketamine (30 mg/kg) and xylazine (2.5 mg/kg). Then, the eyes were enucleated and fixed in 4% Gluteraldehyde in sodium cocodylate buffer for 4 hand transferred to 1% osmium tetroxide and dehydrated through a graded ethanol series. The specimens were embedded in resin. Semi thin and ultrathin sections of the retina were stained with toluidine blue for semi thin sections and lead citrate and uranyl acetate for ultrathin sections. The morphometric study was examined by micrometrical technique in light microscope and ultrathin sections were evaluated by transmission electron microscope (Philips CM-10, Eindhoven, Netherlands). The statistical analyses were performed by using one way Anova. P<0.05 was considered statistically significant.

**Ethical approval**

All procedures and care of the animals were conducted following protocols approved by the ethical committee (Iranian Society for the Prevention of Cruelty to Animal, and Iranian Veterinary Organization).

**Results**

Clinical animadversion showed that scarring and edema were seen in eyelid after formaldehyde injection. The ultra-structural study of photoreceptor layer in control group showed that outer segments contain bimembranous discs like a ladder. The inner
segments included mitochondria, endoplasmic reticulum. The mitochondria near the outer segments and oval to rounded shape were observed (Figure 1). The cristae of mitochondria were sheet-like invaginations of the inner membrane into the matrix (Figure 1). The outer nuclear layer consisted of rod and cone nuclei with heterochromatin (Figure 2). The major pathological signs were seen in the photoreceptor layer in formaldehyde group. Disorganized and vacuolated outer segments, highly vacuolated inner segment, loss of cristae in mitochondria, pyknotic and karyolysis nuclei were evident after the use of retrobulbar injection of formaldehyde (Figures 3, 4). But the photoreceptor layer of erythropoietin injected group has obviously changed from the formaldehyde group. Minor signs of pathology appeared in erythropoietin group, with evidence of lightly vacuolated inner segment (Figure 5). The normal and organized outer segments, normal outer nuclear layer and normal mitochondria were evident in this group (Figures 5, 6). Morphometric study of the photoreceptor layer indicated that the mean thickness of photoreceptor layer in control, formaldehyde and erythropoietin groups was $83.3 \pm 0.83$ micrometer, $73.24 \pm 0.82$ micrometer and $82 \pm 1.58$ micrometer, respectively. The morphometric of photoreceptor layer showed that the thickness of this layer decreased in formaldehyde group with significant difference compared with control group ($p \leq 0.05$) and increased in erythropoietin group with no significant difference compared with control group ($p \geq 0.05$).

**Figure 1:** Electro micrograph of the photoreceptor layer in control group. The outer segment (arrows), the mitochondria in inner segment (thick arrows) (staining with lead citrate and uranyl acetate) ($\times 6600$).

**Figure 2:** Electro micrograph of the photoreceptor layer in control group. The outer segment (OS), the inner segment (IS), the outer nuclear layer (ONL). (Staining with lead citrate and uranyl acetate) ($\times 2950$).
**Figure 3:** Electro micrograph of the photoreceptor layer in formaldehyde group. The vacuolated and disorganized outer segment (arrows), the vacuoles in inner segment (arrowheads), the destroyed mitochondria without cristae (thick arrows). (Staining with lead citrate and uranyl acetate) (× 6600).

**Figure 4:** Electro micrograph of the photoreceptor layer in formaldehyde group. The highly vacuolated inner segment (IS) the vacuoles (arrowheads), the outer nuclear layer (ONL), pyknotic nuclei (thick arrows) and karyolysis (wave arrow). (Staining with lead citrate and uranyl acetate) (× 4500).

**Figure 5:** Electro micrograph of the photoreceptor layer in erythropoietin group. The normal outer segment (arrow), the normal mitochondria in the organized inner segment (thick arrows), vacuoles in inner segment (arrowheads) (Staining with lead citrate and uranyl acetate) (× 6600).

**Figure 6:** Electro micrograph of the photoreceptor layer in erythropoietin group. The normal mitochondria in the organized inner segment (thick arrows), vacuoles in inner segment (arrowheads), the normal nuclei in outer nuclear layer (arrow) (Staining with lead citrate and uranyl acetate) (× 6600).
Discussion
The photoreceptor layer of the rat is similar to that of other animals (Esfandiari et al, 2009; Goodarzi et al, 2014; Garcia & Dejuan, 1999; Haacke et al, 2001). In this research, the injury produced by formaldehyde injection and improvement by erythropoietin injection were evaluated by transmission electron microscope. The rats injected with formaldehyde showed disorganization and vacuolization of the outer segment, high vacuolization of the inner segment, disappearance of cristae of mitochondria, pyknotic and karyolysis in outer nuclear layer. These histopathological changes were seen in formaldehyde group whereas the formaldehyde caused ischemia due to central retinal and ophthalmic arteries occlusion (Soltansanjari & Hashemi, 2004). In addition, the formaldehyde enhanced reactive oxygen species as a result of inflammation (Turkoglu et al, 2008). The cell injury and damage occurred as a result of increasing reactive oxygen species. On the other hand, ischemia increases oxygen free radical, nitric oxide and glutamate levels (George & Cioffi, 2005). However, these damages and injuries of the photoreceptor layer corroborated prior research, showing that retinal ischemia increased apoptosis (Matsuka et al, 2010; George & Cioffi, 2005; Wyllie, 1997). By comparison, the normal outer segment, inner segment with light vacuolization and the normal outer nuclear layer were seen in erythropoietin group. Increasing erythropoietin for survival may limit neuronal damage in cerebral and retinal ischemia (Siren et al, 2001; Bernaudin et al, 1999; Junk et al, 2002; Kawakami et al, 2001). Exogenous erythropoietin has been shown to decrease damage of ischemia in brain injury and inflammation (Siren et al, 2001; Bernaudin et al, 1999). Our finding corroborated the previous studies (Siren et al, 2001; Bernaudin et al, 1999; Junk et al, 2002; Kawakami et al, 2001). Rats given intraperitoneal erythropoietin immediately after the formaldehyde injection for 7 days reduced photoreceptor damage. This finding demonstrates that post treatment with erythropoietin leads to improvement of neuronal damage of ischemia. The mechanisms may explain how erythropoietin improves neuronal injury. The erythropoietin has an antiapoptotic role due to reduction of TUNEL-positive cells in the cerebral ischemia (Siren et al, 2001). Also, erythropoietin inhibited apoptosis by depriving growth factor (Siren et al, 2001; Bernaudin et al, 1999). In addition, the erythropoietin inhibited the release of glutamate from neuron (Kawakami et al, 2001). On the other hand, ischemia increased glutamate (George & Cioffi, 2005) but erythropoietin decreased glutamate (Kawakami et al, 2001) and attenuates neuronal damage after exposure to the glutamate (Sinor & Greenberg, 2000). Our observation is confirmed by prior research showing that erythropoietin can decrease neuronal injury (Brines et al, 2000; Coleman et al, 2006). Also, the erythropoietin can be delivered to a damaged tissue; this treatment can affect increases in hematocrit with associated vascular occlusion (Brines et al, 2000; Coleman et al, 2006). Our finding in the outer nuclear layer of the formaldehyde group suggests that there are pyknotic and karyolysis nuclei. But erythropoietin can inhibit neuronal cell apoptosis (Grimm et al, 2002), and clean oxygen radicals of ischemia (Marti et al, 2000) against oxidative stress after ischemia (Wang et al, 2010). Our findings are consistent with the results of previous studies (Grimm et al, 2002; Marti et al, 2000; Wang et al, 2010). On the other hand, reduction of ATP produced and cessation of oxidative phosphorylation occurred in hypoxia (Macario & Conway de Macario, 2000). Depletion of ATP with decreasing of PH, increased influx of Na and Ca ions occurred in ischemia and anaerobic metabolism (Uysal et al, 2014; Thomaz Neto et al, 2013). In our research, morphological changes and loss of cristae
of mitochondria in inner segment of retina have seen and confirmed by previous study indicating that ATP reduction and increase mitochondrial permeability transition pore in ischemia (Uysal et al, 2014; Thomaz Neto et al, 2013). Also, efflux K ion and influx Na ion with water caused cellular edema in hypoxia (Chambless et al, 2003). The edema caused damage the cells and cytoplasmic organelles such as mitochondria (Esfandiari et al, 2009). These ultra-structural observations confirmed that the improvement effect of erythropoietin in damaged photoreceptor cell after ischemic induced by formaldehyde exposure. The proposed study will be the intracellular pathway associated with cell survival and apoptosis under the effect of erythropoietin and formaldehyde.

Conclusions
The results of our research concluded that the erythropoietin could increase the recovery of neuronal function after ischemia damage. Also, our observation demonstrates that erythropoietin inhibit apoptosis after ischemia and may use a therapeutic agent for retinal ischemia disease such as glaucoma or vascular occlusion.

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