The Protective Effect of Latanoprost on the Ischemia/Reperfusion Injury in the Retina of Albino Rat: Light and Electron Microscopic Studies

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بسم الله الرحمن الرحيم

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Abstract

Background: Retinal ischemia is a common clinical entity and it is a common cause of visual impairment and blindness. Thus, increased understanding of the events involved in ischemic neuronal injury can provide us with clinically effective treatments for many retinal diseases.

Aim of the work: This study was conducted to demonstrate the histopathological effects of the induced retinal ischemia/reperfusion injury in the albino rats and the potential protective effect of latanoprost eye drops.

Material and Methods: Fourty two adult male albino rats were divided into two groups: control group (n=12) and experimental group (n=30), further subdivided into 6 subgroups. Ischemia was induced in the left eyes of the first three subgroups by increasing the intra-ocular pressure; they were sacrificed after 60 minutes, 24 hours and 7 days respectively. Latanoprost was applied to both eyes of the animals of the other three subgroups once daily for 1 week, at the end of the treatment, ischemia was induced in the left eyes and they were sacrificed as discussed before. The retinal tissues were processed and investigated by light and electron microscopy. Morphometric studies followed by statistical analysis of the number of the cells in the GCL were performed.

Results: The untreated subgroups showed marked morphological changes demonstrated in the different retinal layers. The PRL showed separation, vacuoles and degeneration. The ONL and INL showed marked nuclear condensation and separation between nuclei. Vaculations &disruption could be seen in the IPL, GCL, NFL and ILM. The treated subgroups showed more or less preserved retinal layers.

Conclusion: It is concluded that the ischemia induced degenerative damage in all retinal layers. Moreover, the reperfusion aggravated the ischemic damage. In addition, the retina showed persistent damage upon recovery. Also, it is concluded that latanoprost acts partially as a protective agent against the induced retinal I/R injury.

Key words:

Ischemia	Latanoprost
Degeneration	Retina

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To my parents,
To my husband,
To my children,
To all my family,
Thank you with all
the love and gratitude.

LIST OF ABBREVIATIONS

µg	microgram
ANOVA	Analysis of Variance
bFGF	basic Fibroblast Growth Factor
BRAO	Branch Retinal Artery Occlusion
BRB	Blood Retinal Barrier
CC	Connecting Cilium
CRA	Central Retinal Artery
CRAO	Central Retinal Artery Occlusion
CRVO	Central Retinal Vein Occlusion
ERG	Electroretinogram
GCL	Ganglion Cell Layer
HIF-1	Hypoxia Inducible Factor - 1
I/R	Ischemia / Reperfusion
INL	Inner Nuclear Layer
IOP	Intra Ocular Pressure
IPL	Inner Plexiform Layer
IS	Inner Segment
MGC	Muller Glia Cell
NO	Nitric Oxide
ONL	Outer Nuclear Layer

OPL	**Outer Plexiform Layer**
ORFs	**Outer Retinal Folds**
OS	**Outer Segment**
PEDF	**Platelet Endothelial Derived Factor**
PRL	**Photoreceptor Layer**
PGF2-alpha	**Prostaglandin F2-alpha**
POBF	**Pulsatile Ocular Blood Flow**
pRGCs	**photosensitive Retinal Ganglion Cells**
RBCs	**Red Blood Corpuscles**
RGC	**Retinal Ganglion Cell**
RPE	**Retinal Pigment Epithelium**
ROS	**Reactive Oxygen Species**
RPCs	**Radial Peripapillary Capillaries**
SD	**Standard Deviation**
SPSS	**Statistical Package for the Social Sciences**
ST	**Synaptic terminal**
TEM	**Transmission Electron Microscope**
VEGF	**Vascular Endothelial Growth Factor**

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Retinal ischemia is a common clinical entity and, due to relatively ineffective treatment, remains a common cause of visual impairment and blindness. It is a characteristic feature of various clinical retinal disorders such as ischemic optic neuropathies, obstructive arterial and venous retinopathies, carotid occlusive disorders, retinopathy of prematurity, chronic diabetic retinopathy and glaucoma **(Osborne et al., 2004)**.

A more thorough understanding of the molecular mechanisms behind ischemic damage is essential to improve potential therapies and can provide insight into the pathophysiology of other neurodegenerative conditions as well (**Baker et al., 2008)**.

The retina is supplied by two arterial systems: the choroiocapillary plexus supplies the outer retina, while the central retinal artery supplies the inner retina. The rich capillary networks provide an excellent blood supply suiting the high energy demand of the retinal light processing events **(Feigl, 2009)**.

Ischemic retinopathy develops when retinal blood flow is insufficient to match the metabolic needs of the retina **(Osborne et al., 2004)**. As a result of retinal ischemia, new blood vessels are formed to meet the metabolic demands of the ischemic tissue. These newly formed blood vessels malfunction and are unable to supply the tissue with the necessary nutrients. They are not included in the blood retinal barrier and will thus leak and bleed. This leads to sight-threatening complications such as tractional retinal detachment, vitreous hemorrhage, neovascular glaucoma and macular edema **(Dorrell et al., 2007)**.

There is no effective treatment strategy to protect the retina from ischemia-reperfusion damage. Several obstacles have limited the development of neuroprotective agents for retinal ischemia. First, there is the issue of timing; a drug would have to be given within a reasonable time window of the ischemic event. Second, all attempts to date have been characterized by incomplete protection and the risk for nonspecific effects, including toxicity **(Roth, 2004*)***.

One of the most frequently used models for the investigation of molecular mechanisms and potential strategies for retinal ischemia has been an experimental model of acute elevation of the intra ocular pressure followed by reperfusion **(Zheng et al., 2007)**.

Intra ocular pressure lowering treatment, either by the use of topically applied drugs or by surgery, is the only treatment for glaucoma. Among various topical drugs, prostaglandin analogue latanoprost is one of the newest types of glaucoma medications. Latanoprost has powerful ocular hypotensive effects. However, its effect in retinal ischemia as well as ischemia reperfusion is not clearly documented **(Drago et al., 2001)**.

Aim of the Work

The aim of the present work was to study the histopathological effects of acute retinal ischemia induced by elevating the intraocular pressure as well as ischemia followed by reperfusion in adult male albino rats. Moreover, the potential protective effect of prostaglandin analogue latanoprost eye drops was examined.

Embryology of the Retina

The retina is a part of the central nervous system and each eye is formed from a lateral invagination from the neural tube. This invagination or optic vesicle further invaginates to form the optic cup or eyeball **(Sterling and Demb, 2004)**. The outer layer of the optic cup forms a single layer of pigmented cells. Pigmentation begins at the end of the fifth week. The inner layer undergoes a complex differentiation into the nine layers of the neural retina. The photoreceptors (rods and cones) as well as bipolar, amacrine, ganglion cells and nerve fibers are present by the seventh month. The macular depression begins to develop by the eighth month and is completed at the sixth month after birth **(Ross and Pawlina, 2011)**.

Retinal development begins centrally before extending peripherally, i.e., a rudimentary macula is formed first followed by peripheral retina. Subsequent expansion of the peripheral retina accounts for most of the eye's axial growth. At the same time, the retina matures in a centripedal direction, i.e., the peripheral retina is fully developed long before the posterior pole is. Thus the fovea is immature at birth and undergoes maturational changes for several years **(Reh and Moshiri, 2006).**

Cellular proliferation expands retinal tissue mass, experiments in rodents have shown that retinal stem cells are capable of producing all retinal neuron cell types and Müller cells **(Boyd and Matsubara, 2003).**

Functional Histology of the Retina

The mammalian retina is the innermost layer of the globe forming the nervous coat of the eye. It is a thin, delicate layer; its thickness is 0.2 mm. It consists of two components:

* The **neural retina** or **retina proper**, an inner layer that contains light sensitive receptors (rods &cones) and complex neuronal networks.

* The **retinal pigment epithelium**, an outer layer composed of simple cuboidal melanin-containing cells. A potential space exists between these two layers of the retina. The two layers may be separated mechanically in the preparation of the histological specimens. Separation of the layers, retinal detachment, also occurs in the living state as a result of eye diseases or trauma **(Ross and Pawlina, 2011)**.

Externally, the retina rests on choroid; internally it is associated with the vitreous body. Visual information encoded by rods and cones is sent to the brain via impulses conveyed via the optic nerve **(Ross and Pawlina, 2011)**.

In the retina proper, two regions that differ in function are recognized **(Fig.I)**:

*__The non-photosensitive (nonvisual part)__, located anterior to the **orra serrata**, which lines the inner aspect of the ciliary body and posterior surface of the iris.

*__The photosensitive (optic part)__, which lines the inner surface of the eye posterior to the orra serrata except where it is pierced by the optic nerve. The site where the optic nerve joins the retina is called **optic papilla** or **disc**. Because the optic papilla is devoid of photoreceptors it is a blind spot in the visual field. The **fovea centralis** is a shallow depression located about 2.5 mm lateral to the optic disc. It is the area of the greatest visual acuity. The visual axis of the eye passes through the fovea. A yellow –pigmented zone called **Macula lutea** surrounds the fovea **(Ross and Pawlina, 2011)**.

The rat eye is about 7 mm in diameter, which is approximately 3.5 times smaller in diameter than the human eye. The rat retina does not have a fovea and has a higher proportion of rod photoreceptors than cone photoreceptors. Otherwise the major structures and functions are similar **(Hughes, 1979).**

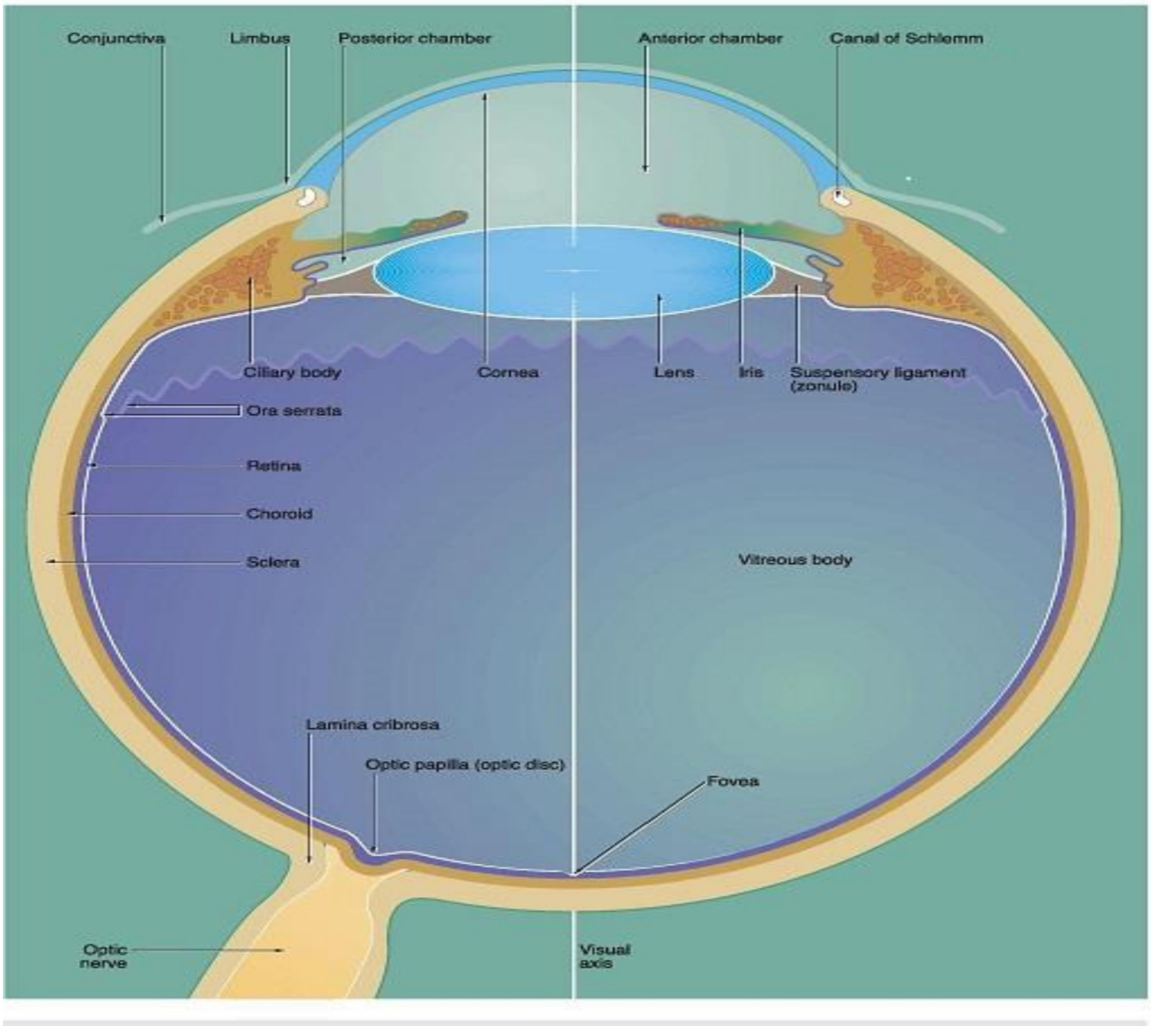

Figure I. Diagram of the Eye (Young et al., 2014).