Effect of Chloroquine Drug on the Retina of Adult Albino Rats

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Abstract

Background and Aim: Chloroquine drug was first used as an antimalarial agent. It subsequently played an important therapeutic role in various rheumatologic diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and other inflammatory and dermatologic conditions. Retinal toxicity from chloroquine has been recognized for decades; however it is still used in third world countries due to its cheap price. So, the present work was carried out to show the effects of chronic administration of chloroquine drug on the retina of adult albino rats.

Material and Methods: In the present work, twelve adult male albino rats, with average weight of 200g were randomly assigned into two groups: Control group (n=6) and Treated group (n=6). The rats in the treated group received 19mg/day of chloroquine drug for three months. The drug was administered directly into the stomach through oro-gastric tube. The control group received equal volume of distilled water through the same route. The rats were sacrificed by cervical dislocation method and the eyeball was carefully enucleated out and the specimens were cut into small parts (each was about 1X 2mm). The samples were quickly fixed in 10% formal saline for histological study.

Results: In the present study, results of semithin sections stained with toluidine blue showed reduction in the retinal thickness of the experimental animals which affected mainly the outer nuclear layer. The inner cell layer of the experimental retinae showed distortion of the bipolar and amacrine cells with vacuoles in their cytoplasm. The ganglionic cells of the experimental retinae showed distorted nuclei and cytoplasmic vacuoles when compared with the control retinae. In paraffin sections stained with gallocyanin dye, the experimental retinae showed distorted nuclei and cytoplasmic vacuoles when compared with the control retinae. There was a highly significant decrease in the thickness of the bipolar cell layer thickness (p=0.155). The experimental retinae had a highly significant decrease in the numerical densities of the photoreceptor cell layer (p=1.9E-25) when compared with the control retinae but there was no significant change in the numerical densities of the bipolar cell layer (p=0.13).

Conclusion: This study revealed that chronic administration of chloroquine can cause micro-anatomical changes in the retinae of the adult albino rats. This histological effect may provoke cognitive dysfunction as well as affect the visual sensibility functions of the retinae. It is recommended that further studies aimed at corroborating these observations be carried out.

Key Words: Chloroquine – Retina – Albino rats.

Introduction

CHLOROQUINE was first used as an antimalarial agent. It subsequently played an important therapeutic role in various rheumatologic diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and other inflammatory and dermatologic conditions. Retinal toxicity from chloroquine has been recognized for decades; however it is still used in third world countries due to its cheap price [1]. Chloroquine is a lysosomotropic antimalarial drug that is widely used for treatment of muco-cutaneous and musculoskeletal problems in many connective tissue diseases such as, rheumatoid arthritis, systemic lupus erythematosus, and scleroderma [2]. The drug is rapidly absorbed after oral administration, and distributes extensively in body tissues, particularly in pigmented eye tissue, resulting in ocular toxicity. Chloroquine associated ocular side effects include accommodation abnormality, which is the most common; corneal deposition, and pre and true retinopathy. The accommodation defect, corneal deposition, and pre-retinopathy are in general completely reversible with drug discontinuation [3]. On the other hand, “Bull’s eye retinopathy” is a major concern as it has irreversible damage [4].
Patients usually complain of color vision disturbances, blurring of vision and field defects despite dose limitation and ophthalmologic monitoring, irreversible retinal damage can occur [5].

The mechanisms of action of chloroquine are not clearly understood. Chloroquine can interfere with intracellular functions, inhibits enzyme activity, has anti-inflammatory activity, and effects on immune functions [6].

Thus, the aim of the present study was to detect the histological and ultrastructural retinal changes induced by chronic chloroquine administration.

Material and Methods

In the present study, 12 adult male albino rat retinæ were used. The average weight of the animal was 200 grams. The animals were housed in the animal house of the Faculty of Medicine, Assiut University in a normal daily light and darkness cycle. The animals were fed with normal show and water. The animals were divided into two groups with 6 animals in each group.

1- Non-treated group: This group consisted of six animals. The animals were one month of age. These animals were taking one ml of water every day for 3 months.

2- Chloroquine treated group: This group consists of six animals. The animals were one month of age. The animals were taking one ml of drug suspension for 3 months.

Drug dosage and administration:

The dose in the present study was 19mg/day for three months according to Duncker et al.. [7]. The drug was administered directly into the stomach by an oro-gastric tube.

Enucleation of the eyeball was done by cutting the conjunctiva near the conjunctivo-scleral junction. The specimens were cut into small parts (each was about 1 X 2mm). The samples were taken from the mid-central part of the retina.

Tissue preparation:

The tissues were prepared for light microscopic examination according to Drury and Wallington, [8].Gallicyanine chrom-alum staining method was used to stain the slides. Also, the tissues were processed for electron microscopic examination [9]. Two types of section cutting were used:

- Semithin section (1 micron): The sections were stained by 2% aqueous toluidine blue, the sections were used for morphometry.

- Ultrathin sections (0.1 micron): Ultrathin sections were stained with uranyl acetate and lead citrate and sections were examined by using transmission electron microscope in the Electron Microscope Unit, Assiut University.

Stereological procedures:

A number of non-overlapping diagrams were made by Camera Lucida (Leitz Wetzlar, Germany) using a Leitz light research microscope. These diagrams were drawn for stereological procedures.

A digitizing set consisted of Digitizer KD 3040 B connected to IBM compatible personal computer, was used with a specially prepared program to measure lengths. The major diameter (a), which is the widest diameter and minor diameter (b), which is the widest diameter perpendicular over (a). The diameter of equivalent circle (D₀) was calculated (D₀ = ab), Schwartz-Saltikov correction procedure (appendix 1) was applied to obtain more reliable estimates of the true mean nuclear diameter (D̂₀).

The following parameters were calculated for each animal group:

- The total retinal thickness, photoreceptor cell layer thickness and the bipolar cell layer thickness (t).

- The numerical density (Nv) of the photoreceptor cells and the bipolar cells. The numerical density per unit volume of the retina was calculated as follow:

\[ Nv = \sqrt{\frac{N}{aD^- + d}} \]

Where

N : The number of calculated cells.

a : The area in which the number of calculated cells were measured.

D^- : The corrected mean diameter of the nucleus.

The mean value, the standard deviation and the standard error were calculated for each parameter. Unpaired student t-test was used to compare between the mean values of different groups.

Results

Results of light microscope examination:

In the semithin sections stained with toluidine blue: There was a reduction in the retinal thickness of the experimental animals when being compared to the control one. The reduction affected mainly the outer nuclear layer (Plate 1, Figs. 1,2). The inner cell layer of the experimental retinæ showed distortion of the bipolar and amacrine cells with vacuoles in their cytoplasm (Plate 1, Figs. 3,4).
The ganglionic cells of the experimental retinas showed distorted nuclei and cytoplasmic vacuoles when compared with the control retinas (Plate 2, Figs. 1,2).

**Fig. (1):** A photomicrograph of a semithin section of the control albino rat retina showing:
- E: Retinal Pigmented Epithelium.
- F: Bacillary Layer.
- ONL: Outer Nuclear Layer.
- OPL: Outer Plexiform Layer.
- INL: Inner Nuclear Layer.
- IPL: Inner Plexiform Layer.
- GL: Ganglionic Cell Layer.

In paraffin sections stained with gallocyanin dye (Plate 2, Figs. 3,4): The experimental retinas showed a decrease in the staining affinity to the dye with reduction in the whole retinal thickness.

**Fig. (2):** A photomicrograph of a semithin section of the experimental albino rat retina showing:
- E: Retinal Pigmented Epithelium.
- P: Bacillary Layer.
- ONL: Outer Nuclear Layer.
- OPL: Outer Plexiform Layer.
- INL: Inner Nuclear Layer.
- IPL: Inner Plexiform Layer.
- GL: Ganglionic Cell Layer.

**Fig. (3):** A photomicrograph of a semithin section in the inner nuclear layer of the control albino rat retina showing:
- Horizontal cells (H) with large pale nuclei.
- Bipolar cells (B) with darker nuclei.
- Muller’s cells (M) with angular nuclei.
- Amacrine cells (A) with large nuclei.

**Fig. (4):** A photomicrograph of a semithin section in the inner nuclear layer of the experimental albino rat retina showing:
- Distorted bipolar cells nucleus (B).
- Cytoplasmic vacuoles (V).
Fig. (1): A photomicrograph of a semithin section in the ganglion cell layer of the control rat retina showing:

* A large pale ganglionic cell (GC) with large nuclei.

Toluidine blue X1000

Fig. (2): A photomicrograph of a semithin section in the ganglion cell layer of the experimental albino rat retina showing:

* Shrunken nucleus of ganglion cell (N) with cytoplasmic vacuoles (V).

Toluidine blue X1000

Fig. (3): A photomicrograph of a midsagittal section of the experimental rat retina showing:

* Bacillary Layer (P).
* Outer Nuclear Layer (ONL).
* Outer Plexiform Layer (OPL).
* Inner Nuclear Layer (INL).
* Inner Plexiform Layer (IPL).
* Ganglion Cell Layer (GL).

Enirson’s Gallocyanine X400

Fig. (4): A photomicrograph of a midsagittal section of the control rat retina showing:

* Bacillary Layer (P).
* Outer Nuclear Layer (ONL).
* Outer Plexiform Layer (OPL).
* Inner Nuclear Layer (INL).
* Inner Plexiform Layer (IPL).
* Ganglion Cell Layer (GCL).

Enirson’s Gallocyanine X400
Fig. (1): An electron photomicrograph of a section in the outer nuclear layer of the control albino rat retina showing:
- Dense chromatin of the nucleus (N).

Fig. (2): An electron photomicrograph of a section in the outer nuclear layer of the experimental albino rat retina showing:
- Dense chromatin of the nucleus (N).
- Cytoplasmic vacuoles (V).

Fig. (3): An electron photomicrograph of a section in the outer segment of the bacillary layer of the control rat retina showing:
- Lamellar structure (L).

Fig. (4): An electron photomicrograph of a section in the outer segment of the bacillary layer of the experimental rat retina showing:
- Cytoplasmic vacuoles (V).

Fig. (5): An electron photomicrograph of a section in the inner nuclear layer of the control albino rat retina showing:
- Bipolar cells (B).
- Muller’s cells (M).

Fig. (6): An electron photomicrograph of a section in the inner nuclear layer of the experimental albino rat retina showing:
- Distorted bipolar cell nucleus (B) and Shrunken nucleus of Muller’s cell (M).
- Cytoplasmic vacuoles (V).
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Plate (4)

Fig. (1): An electron photomicrograph of a section in the ganglionic cell layer of the experimental rat retina showing:
- Swollen mitochondria (M) with loss of structural details.
- Lipofuscin granules (LF) and Cytoplasmic vacuoles (V).

Results of electron microscope examination:
There was degeneration in the outer segments of the experimental retinae with loss of its lamellar pattern when compared to the control retinae (Plate 3, Figs. 3,4). The outer nuclear layer showed degenerated shrunken nuclei with cytoplasmic vacuoles (Plate 3, Figs. 1,2). The bipolar cells and Muller’s cells had distorted nuclei with cytoplasmic vacuoles (Plate 3, Figs. 5,6). The cytoplasm of the ganglionic cells of the experimental retinae when compared to the control ones showed cytoplasmic vacuoles, lipofuscin granules and swollen mitochondria in the experimental animals (Plate 4, Figs. 1,2).

Results of stereological analysis:
On comparing the control and the experimental retinae, Table (1) and Chart (1) showed a highly significant decrease in the total retinal thickness (212.6 µm ± 3.39 µm Vs 176.2 µm ± 3.25 µm, p=1.87E-31) and the photoreceptor cell layer thickness of the experimental ones (55.4 µm ± 1.01 µm Vs 44.7 µm ± 1.4 µm, p=3.12E-27). On the other hand, there was no significant change in the thickness of the bipolar cell layer thickness of the experimental retinae (31.63 µm ± 0.52 µm Vs 31.47 µm ± 0.46 µm, p=0.155).

Table (2) and Chart (2) showed that the experimental retinae had a highly significant decrease in the numerical densities of the photoreceptor cell layer (4198.2 X 10³ ± 122.5 X 10³ Vs 2966.03 X 10³ ± 177.5 X 10³, p=1.9E-25) when compared with the control retinae but there was no significant change in the numerical densities of the bipolar cell layer (896.9 X 10³ ± 43.7 X 10³ Vs 880.7 X 10³ ± 43.1 X 10³, p=0.13).

Table (1): The means (X) ± standard deviations (SD) of the total retinal thickness, photoreceptor cell layer thickness & bipolar cell layer thickness measured in the control and experimental retinae.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean total retinal thickness X ± SD</th>
<th>Mean photoreceptor cell layer thickness X ± SD</th>
<th>Mean bipolar cell layer thickness X ± SD</th>
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<tbody>
<tr>
<td>Animal Groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>212.6 µm ± 3.39 µm</td>
<td>55.4 µm ± 1.01 µm</td>
<td>31.63 µm ± 0.52 µm</td>
</tr>
<tr>
<td>N = 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental</td>
<td>176.2 µm ± 3.25 µm</td>
<td>44.7 µm ± 1.4 µm</td>
<td>31.47 µm ± 0.46 µm</td>
</tr>
<tr>
<td>N = 6</td>
<td></td>
<td></td>
<td>0.155</td>
</tr>
<tr>
<td>Control Vs Exp.</td>
<td>1.87E-31**</td>
<td>3.12E-27**</td>
<td></td>
</tr>
</tbody>
</table>

(**) Highly significant. (N) Number of the animals per group.
Table (2): The means (X) ± standard deviations (SD) of numerical densities of the photoreceptor cell layer & the numerical densities of the bipolar cell layer measured in the control and experimental retinae.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean numerical densities of the photoreceptor cells</th>
<th>Mean numerical densities of the bipolar cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal Groups</td>
<td>X ± SD</td>
<td>X ± SD</td>
</tr>
<tr>
<td>Control</td>
<td>4198.2 X 10^3 ± 122.5 X 10^3</td>
<td>896.9 X 10^3 ± 43.7 X 10^3</td>
</tr>
<tr>
<td>Experimental</td>
<td>2966.03 X 10^3 ± 177.5 X 10^3</td>
<td>880.7 X 10^3 ± 43.1 X 10^3</td>
</tr>
<tr>
<td>Control Vs Exp. (p-value)</td>
<td>1.9E-25**</td>
<td>0.13</td>
</tr>
</tbody>
</table>

(**) Highly significant. (N) Number of the animals per group.

Discussion

The present study was carried out to determine the alterations in the retinal cells chronically exposed to chloroquine. Chloroquine-induced retinal toxicity has been reproduced in several animal species, including rat, cat, dog, rabbit, pig, and monkey [10-15]. There is very limited, yet contrasting literature on the effects of chloroquine therapy has been implicated to possess retinal toxicity [16]. Morphological hallmark is the intracellular accumulation of membranous phospholipid inclusions (myeloid bodies) that occur mainly in photoreceptors and retinal pigment epithelium (RPE) and (apparently to a lesser degree) in ganglion cells [7,12,14,17,18].

RPE cells largely remain intact, but eventually the photoreceptor outer segment (POS) deteriorates [18]. Although it has been speculated that oxidative stress is a primary mechanism of chloroquine-induced retinal toxicity [19,20], there is extensive evidence suggesting that chloroquine primarily inhibits lysosomal degradation [21-25]. The high affinity of chloroquine for melanin and its potential to accumulate within the eye are thought to be an important pathogenic mechanism, but its significance is still obscure [26].

The results of the semithin sections stained with toluidine blue stain from this experiment revealed some cellular degenerative changes such as sparse cellular population, pyknotic nuclei in the stroma of the rat retinae, when compared to the control group.

In the present study, analysis of the stereological results showed a highly significant decrease in the total retinal thickness and the photoreceptor cell layer thickness and non significant change in the thickness of the bipolar cell layer thickness of the experimental retinae. This is in accordance with the results of Patrick et al., [25] who attributed the affection of photoreceptor cells mainly due the high affinity of chloroquine for melanin and its potential to accumulate within these cell pigment molecules.

Similarly, the stereological results of the present study showed that the experimental retinae had a highly significant decrease in the numerical densities of the photoreceptor cell layer but there was no significant change in the numerical densities of the bipolar cell layer. Anderson et al., [27] reported that Chloroquine has an affinity for pigmented (melanin-containing) structures. They added that melanin serves as a free-radical stabilizer and as an agent that can bind toxins. With more pro-
longed exposure, the drug accumulates mainly in the photoreceptor cell layer of retina. The drug is retained in the pigmented structures long after its use is stopped.

In the present study, chloroquine may have acted as toxin to the cells of the rat retinae, affecting their cellular integrity and causing defect in membrane permeability and cell volume homeostasis [28]. In cellular necrosis, the rate of progression depends on the severity of the environmental insults. These may be substances present in small amounts in the environment, or even naturally occurring chemicals such as glutamate used as transmitter’s substances. The latter when present at a critical level can be toxic to the retina cells in which they normally excite [29].

It could be inferred from this study that prolonged administration of chloroquine resulted in increased toxic effects on the rat retina. That is, the decrease in cellular population observed in this study may have been as a result of cell death caused by the toxic effect of chloroquine. In the same way, it has been reported that chronic administration of chloroquine resulted in cellular degenerative changes, sparse cellular population and vacuolation appearing in the stroma with some autophagic vacuoles in the rat retina [30,31].

Mahon et al. [23] and Yamada et al. [26] reported that chloroquine, which was a weak base, neutralized the acidic organelles such as lysosomes. This results in elevation of its PH with subsequent inhibition of their acid dependent hydrolases.

What this article contributes to literature is that the toxic effect of chloroquine may be at the micro-anatomical level of the retina. It is probable that observable vision defect attributable to chloroquine would be dependent on the extent of effect on the micro-anatomical structure of the retina.

Conclusion:

The present study revealed that chronic administration of chloroquine can cause microanatomical changes in the retinae of the adult albino rats. This histological effect may provoke cognitive dysfunction as well as affect the visual sensibility functions of the retinae in the adult albino rats. A further study to corroborate this finding is wanted.

References


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