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Effect of chronic administration of sildenafil citrate (Viagra) on the histology of the retina and optic nerve of adult male rat

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ABSTRACT

Background: Abnormal vision has been reported by 3% of patients treated with sildenafil citrate (Viagra). Although many men use Viagra for an extended period for treatment of erectile dysfunction, the implications of the long term-daily use of it on the retina and optic nerve are unclear.
Aim of the work: To investigate the effect of chronic daily use of sildenafil citrate in a dose equivalent to men preferred therapeutic dose on the histology of the retina and optic nerve of adult male rat.
Material & methods: Eighteen adult male *Wistar* rats were equally divided into three groups. Group I: control. Group II: treated with sildenafil citrate orally (10 mg/kg/day) for 8 weeks. Group III (withdrawal): treated as group II and then left for 4 weeks without treatment. Specimens from the retina and optic nerve were processed for light and electron microscopy.
Results: In sildenafil citrate treated group, the retina and optic nerve revealed vacuolations and congested blood capillaries with apoptotic endothelial and pericytic cells, and thickened basal lamina. Caspase-3 (apoptotic marker) and CD31 (endothelial marker) expression increased. Glial cells revealed morphological changes: Müller cells lost their processes, activated microglia, astrocytic clasmatodendrosis, degenerated oligodendrocytes surrounded by disintegrated myelin sheathes of the optic nerve fibers. The retina and optic nerve of the withdrawal group revealed less vacuolations and congestion, and partial recovery of the glial cells.
Conclusion: Chronic treatment with sildenafil citrate (Viagra) caused toxic effect on the structure of the retina and optic nerve of the rat. Partial recovery was observed after drug withdrawal.

1. Introduction

Sildenafil citrate (Viagra), a potent inhibitor of the vascular-associated enzyme phosphodiesterase type 5 (PDE5), is used to treat erectile dysfunction which is a serious medical and social problem that occurs in 10%–52% of men (Tharakan and Manyam, 2005). Sildenafil citrate has been shown to be useful for the prevention and treatment of high altitude sickness and pulmonary arterial hypertension (Oliver et al., 2006). It induces vasodilatation by enhancing the smooth muscle relaxant effect of nitric oxide (Grunwald et al., 2001). Nitric compounds produce an increase in optic nerve head circulation and retinal venous vasodilatation (Grunwald et al., 2001). Also, Viagra inhibits phosphodiesterase type 6 (PDE6), an essential enzyme involved in the activation and modulation of the photo transduction cascade, the process by which photons of light are absorbed and converted into electrical signals for transmission to the visual centers of the brain (Stockman et al., 2007). PDE6 is used in the cones cells of the retina; therefore, PDE6 inhibitors may have an effect on color vision (Estrade et al., 1998). Abnormal vision described as a blue tinge to vision or an increase brightness of light, has been reported by 3% of patients treated with sildenafil (Morales et al., 1998; Stockman et al., 2007). Some investigators supported that, PDE6 enzyme inhibition causes dose dependant effects of visual disturbance in men (Sponsel et al., 2000; Gabrieli et al., 2001). Although, there have been reports of chorioretinopathy, serous macular detachment and non-arteritic anterior ischaemic optic neuropathy (NAION) in users of PDE6 inhibitors (Eweka and Eweka, 2010), moreover, some cases of blindness have been attributed to NAION due to misuse of sildenafil citrate (Akash et al., 2005). Tests for visual performance, such as visual acuity and color discrimination are not particularly well suited for monitoring visual effects that are likely to be caused by the inhibition of PDE6.

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As sildenafil citrate is a treatment, not a cure, for erectile dysfunction, many men may choose to use it for an extended period (McMurray et al., 2007), and the implications of the long term-daily use of it on the retina and optic nerve are unclear (Sponsel et al., 2000; Gabrieli et al., 2001). So, this study was carried out to investigate the effect of chronic daily use of sildenafil citrate (Viagra), in a dose equivalent to men preferred therapeutic dose, on the histology of the retina and optic nerve of adult male rat using light and electron microscope.

2. Material and methods

A total number of 18 adult 5 months old male *Wistar* rats (200 g body weight) were used in this study. They were purchased from the Central Animal House, Faculty of Medicine, Assiut University. All animal procedures were in accordance with the standards set forth in the guidelines for the care and use of experimental animals by the Committee for the Purpose of Supervision of Experiments on Animals (CPCSEA) and according to the National Institute of Health (NIH) protocol, and approved by the Institutional Ethics Committee of Assiut University. The animals were housed in clean capacious cages under normal day and night cycles and appropriate temperature (25 ± 5 °C), fed rat chow (standard rat pellets) and water *ad libitum*.

2.1. Animal groups

The animals were equally divided into 3 groups: rats in **group I (control)** were given 0.5 ml distilled water orally by a gastric tube once daily for 8 weeks. Rats in **group II (treated)**, were given sildenafil citrate (purchased from Pfizer INC; USA) orally by a gastric tube once daily at a dose of 10 mg/kg (Valente et al., 2003) dissolved in 0.5 ml distilled water for 8 weeks. This dose was approximately equivalent to a dose of 100 mg/day in men when corrected for differences in total body surface area (Ferrini et al., 2007; Reagan-Shaw et al., 2008), which considered as the men preferred therapeutic dose for erectile dysfunction (Morales et al., 1998; Loran et al., 2009). Rats in **group III (withdrawal)** were given sildenafil citrate orally by a gastric tube once daily at a dose of 10 mg/kg dissolved in 0.5 ml distilled water for 8 weeks and then left for withdrawal of sildenafil citrate for 4 weeks.

The animals were anaesthetized with ether, their hearts were exposed, and then perfusion was done.

For light microscope, three rats from each animal group were used and perfused intracardially with 10% formaldehyde solution. After perfusion, the eyes were dissected immediately, the cornea and the lens were removed and the optic nerve was transected 1–2 mm behind the globe. The retina was separated near the optic disc. Specimens from retina and optic nerve were immersed into the 10% formaldehyde solution to continue fixation two more days. Then the specimens were processed for preparation of paraffin blocks. Paraffin sections (5 μm) were cut using a microtome (Leica RM 2125RT, Germany), mounted on glass slides, and every 10th section was stained with hematoxylin-eosin (Drury and Wallington, 1980). Some sections were stained with Mallory's phosphotungstic acid hematoxylin (PTAH) for demonstration of glial fibrils within astrocytes in the optic nerve (Drury and Wallington, 1980).

For electron microscope, three rats from each group were perfused intracardially with 4% glutaraldehyde in cacodylate buffer (pH7.4). Specimens from retina and optic nerve were cut into thin slices (1 × 1 mm) and immersed in 4% glutaraldehyde in cacodylate buffer (pH 7.4) for 24 h and post fixed in 1% osmium tetroxide in phosphate buffer for two hours. Tissues were rinsed in the same buffer, dehydrated with alcohol, cleared with propylene oxide and embedded in Epon-812 substitute (SPI- Pon Araldit Kit, Cat. no. 02635- AB., SPI- chem. USA). For Polymerization, the embedded samples were kept in the incubator at 35 °C, 45 °C and 60 °C for one day each (Gupta, 1983). Semi-thin sections (0.5–1 μm) were cut with glass knives on the ultramicrotome (LKB Bromma 8800 Ultratome^{R} III, 3518, Sweden) and stained with 1% toluidine blue (pH 7.3) for examination on a light microscope (Olympus, Bx50. Model Bx50F-3, SC09160, Tokyo, Japan). Ultrathin sections (50–80 nm) were cut from selected areas of the blocks on a Reichert ultramicrotome (Leica WILDM3Z, 89386, Austria) placed on copper grids (G 300, 3.05 mm, Polaron Equipment Ltd. Watford. England) and contrasted with uranyl acetate and lead citrate. These sections were examined using the transmission electron microscope (Jeol E.M.-100 CX11; Japanese electron optic laboratory, Tokyo, Japan) and photographed at 80 kV.

2.1.1. Immunohistochemical study

Expression of Caspase-3 (apoptotic marker) and CD31 (endothelial marker) platelet endothelial cells adhesion molecule 1 (PECAM1) was detected in formalin- fixed paraffin- embedded sections. Sections (5 μm) were deparaffinized in xylene and rehydrated in alcohol. Sections were boiled in 10 mM citrate buffer, pH 6.0 for 10 min followed by cooling at room temperature for 20 min. The Caspase 3 (CPP32) Ab-4, Rabbit Polyclonal Antibody (Thermo Fisher Scientific, Fremont, CA 94538-7310, USA) was used at 1:100 dilution for 30 min at room temperature. While, CD31/PECAM1 (clone TLD-3A12) (mouse monoclonal Ab, purchased from Novus Biologicals) was used at 1:10 dilution for 2 h at room temperature. Sections were processed according to the manufacture instructions using the universal kit (Ecno Tek HRP Anti-Polyvalent, DAB) (ScyTek Laboratories, Inc. 205 South 600 West Logan, UT 84321 USA). After completion of the reaction, counterstaining was done using Mayer's haematoxylin, dehydrated and cover-slipped using DPX (Oxford laboratory reagents, Bombay, India).

2.1.2. Morphometric study

Using computer-assisted image analysis (Soft Imaging System, AnalySIS-2004, Olympus Company, Tokyo,Japan), the number of ganglion cells per mm^2 was counted in retina H&E stained sections by touch count method, using a X 400 objective lens. The astrocyte cells body area (μm^2) in optic nerve PTAH stained sections was measured by the arbitrary area method using a 100X oil immersion lens. The number of Caspase-3 and CD31 positive cells per mm^2 was counted in retina and optic nerve immuno-stained sections by touch count method, using a 100 X oil immersion lens. Measurements were done in five non overlapping fields in ten randomly chosen sections from three different animals for each group.

2.1.3. Statistical analysis

The morphometric data of each animal group were statistically analyzed using the computer statisticsPrism-5.0 package (GraphPad Software, Inc., San Diego, CA, USA). One-way analysis of variance (ANOVA) followed by Newman-Keuls test as a post-test was employed to compare the three studied animal groups. Unpaired *t*-test was employed to compare the two groups (II&III). The results were expressed as mean ± standard deviation (SD). P-value < 0.05 was considered significant.

3. Results

3.1. Retina

3.1.1. Light microscopy

3.1.1.1. A- Histological results. In **group I (control)**, the retinal layers were well defined (Fig. 1A), from the scleral to vitreal sides, these were: the retinal pigmented epithelium (RPE), the outer (OS) and inner (IS) segments of photoreceptors. The OS was lighter in

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