·Basic Research·

Effects of intraocular rifampicin on retinal ganglion cell structure: a stereological and histopathological study

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Abstract

• **AIM: To determine the histopathological changes of rifampicin applied intravitreally on retinal ganglion cells by means of stereological and histopathological methods.**

• **METHODS: For this study twenty-four New Zealand adult rabbits were divided into four groups (n=6 for each group). 50μg/0.1mL (group 1), 100μg/0.1mL (group 2), 150μg/0.1mL (group 3) and 200μg/0.1mL (group 4), rifampicin were injected into the vitreous of the right eyes of animals, their left eyes were used as control (group 5). After the 28th day of application, animals were anesthetised with xylazine (8mg/kg, IM) and then their eyes were enucleated immediately. Patterns were taken away and eyes were prepared for both stereological and electromicroscopic observation.**

• **RESULTS: Depending on the high dose of rifampicin, some histopathological changes such as cytoplasmic dilatation and damaged membrane were observed on the electromicroscopic level. Using quantitative examination, which was done at the light microscopic level, it was shown that the number of neurons decreased linearly as rifampicin dose increased when compared with the control group.**

• **CONCLUSION: Based on these findings, low-dose rifampicin (50μg/0.1mL) may be useful for treatment of the ocular diseases.**

• **KEYWORDS:** rifampicin; stereology; histopathology; ganglion cells
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INTRODUCTION

Tuberculosis (TB) is a chronic bacterial infection, and remains a global health problem. It affects millions of people each year and ranks as the second leading cause of death from infectious diseases worldwide, after acquired immunodeficiency syndrome (AIDS) caused by human immunodeficiency virus (HIV). The latest estimates are that there were almost 9 million new cases in 2011 and 1.4 million TB deaths (990 000 among HIV-negative people and 430 000 HIV-associated TB deaths)[1-3]. The disease primarily affects the lungs, although it may also affect other organs. The ocular complications of TB, although less common than systemic involvement, are well recognized. The recurrence of TB as a major public health problem raises the patients with ocular complications [4]. The disease has been reported in almost all ocular tissues [5]. The most common manifestation of ocular TB in patients with pulmonary TB is choroiditis. Retinal periphlebitis is rarely caused by direct invasion of tubercle bacilli in the retina.

Retinal TB is usually, but not always, secondary to an underlying choroiditis. Tuberculoprotein hypersensitivity may play a key role in the pathogenesis of phlyctenulosis and Eales disease. Both ocular and orbital TB are usually unilateral [6]. If they are not treated, they will cause serious vision loss. Therefore, the most effective, secure and short-term treatments should be selected and injection of a drug directly to the relevant region is preferred in patients who cannot tolerate systemic therapy and the treatment of a region that does not drain directly into the systemic

circulation [7]. Another direct application form that has been mentioned by researchers is intravitreal drug injection, and it has been shown that this approach is often more efficient in cases of inflammation [8]. However, intravitreal injection is a process that requires great precision, because it is performed with the help of a needle under local anaesthesia. In this process, the needle must be inserted into the vitreous liquid that fills the cavity between the lens and retina, and one must be very careful not to damage the retina.

Some treatment methods which are combined with a series of drugs such as rifampicin, streptomycin ethambutol and isoniazid are generally applied for treatment of TB. Rifampicin has become one of the most important drugs for the treatment of TB and leprosy since it first became commercially available in the 1970s. It is a semi-synthetic derivative of rifamycin, which is a class of broad-spectrum antibiotics derived from the fermentation products of *nocardia mediterranei*. It inhibits nucleic acid (RNA) synthesis. It is also has bactericidal actions against gram-positive bacteria, including *mycobacteria*, and an excellent antistaphylococcal effect[9].

The aim of this study was to estimate the numerical density of ganglion cells in rabbits' eyes injected with rifampicin and to examine these samples histopathologically both light microscopic and electromicroscopic levels to determine the possible effects of rifampicin treatment.

MATERIALS AND METHODS

Materials In this study, 24 New Zealand adult white rabbits weighing between 2 000g and 2 500g were obtained from Ataturk University Experimental Medical Application and Research Centre. Rabbits were divided into four groups, each containing six animals (n=6), as follows: 50μg/0.1mL (group 1), 100μg/0.1mL (group 2), 150μg/0.1mL (group 3) and 200μg/0.1mL (group 4). The rabbits were fed standard laboratory chow and tap water before the experiment and were housed at 22℃ and with stringent lighting controls (14h in light/10h in dark). At first they were anaesthetized with xylazine HCl (8mg/kg) and 0.5% proparacaine was used for topical anaesthesia. The pupils were dilated with 2.5% phenylephrine and 1% tropicamide. Approximately 0.1mL of aqueous humor was removed through a 25-gauge needle. Then the injection was performed with a 27-gauge needle approximately 2-3mm behind the limbus. While 50, 100, 150, 200μg/0.1mL rifampicin were injected in the intravitreal cavity of the right eyes of each group of animals, respectively, their left eyes were used as controls. After 28d animals were anesthetized with xylazine (8mg/kg, IM) and then their eyes were dissected out immediately. This study received ethical approval from the Experimental Research and Application Center of Atatürk University.

Methods

Histological procedures for light microscopy Tissue specimens of 2-3mm were used for stereological examination. All specimens included the three concentric layers of the eye: the tunica fibrosa, tunica vasculosa and tunica nervosa. Each piece of eye was fixed in 10% formalin solution for 48-55h, dehydrated in a graded alcohol series, embedded in paraffin wax and sectioned using a microtome (Leica RM2125RT). Sections of 5μm in thickness were mounted onto glass slides for stereological analysis. To estimate the number of ganglion cells, selected sections were stained with haematoxylin-eosin. In this phase of the application, in the areas that are fallen into the same microscopic magnification, during the Y-axis, field sampling was done and ganglion cells were counted with a camera and a step meter (Kaplan *et al* 2001). Finally, the number of ganglion cells per unit area in all groups was determined.

Histological procedures for electron microscopy For the electromicroscopic examination, eyes were fixed in 3% glutaraldehyde in 0.1moL/L phosphate buffer, post-fixed in 1% osmium tetroxide in 0.1moL/L phosphate buffer, dehydrated in a graded acetone series and transferred to propylene oxide. After dehydration, specimens were embedded in Araldite CY 212. Sections were cut using an ultramicrotome (LKB NOVA, Bromma, Sweden). Then, ultra-thin sections were stained with uranyl acetate and lead citrate. Finally, sections were examined under a Jeol 100 SX electron microscope (Jeol, Tokyo, Japan).

RESULTS

Histopathological Results In the histopathological examination at the electromicroscopic level, the toxic effects of rifampicin were observed at each of the applied doses. While the control group had a normal structure (Figure 1A), the high-dose groups (100, 150, 200μg/0.1mL) dilated vacuolar structures in the neuron cytoplasm, swollen mitochondria, dilatation in the core membrane, membrane integrity corruption and, in the neuron cores, morphological symptoms of necrotic processes like pyknosis, karyolysis and karyorrhexis (Figure 1B-E).

Quantitative Light Microscopic Results In the quantitative analysis at the light microscopic level, the average number of neurons falling in the same microscope magnification field was calculated (Figure 2) as follows: 1) Control group (2.79 neurons/mm^2); 2) In the group syringed with 50μg/0.1mL rifampicin (2.13 neurons/mm^2); 3) 100μg/0.1mL rifampicin (1.50 neurons/mm^2); 4) 150μg/0.1mL rifampicin (1.04 neurons/mm^2); 5) 200μg/0.1mL rifampicin (0.59 neurons/mm^2). At the light of these results; there were significantly differences between control and experimental groups (P<0.05).

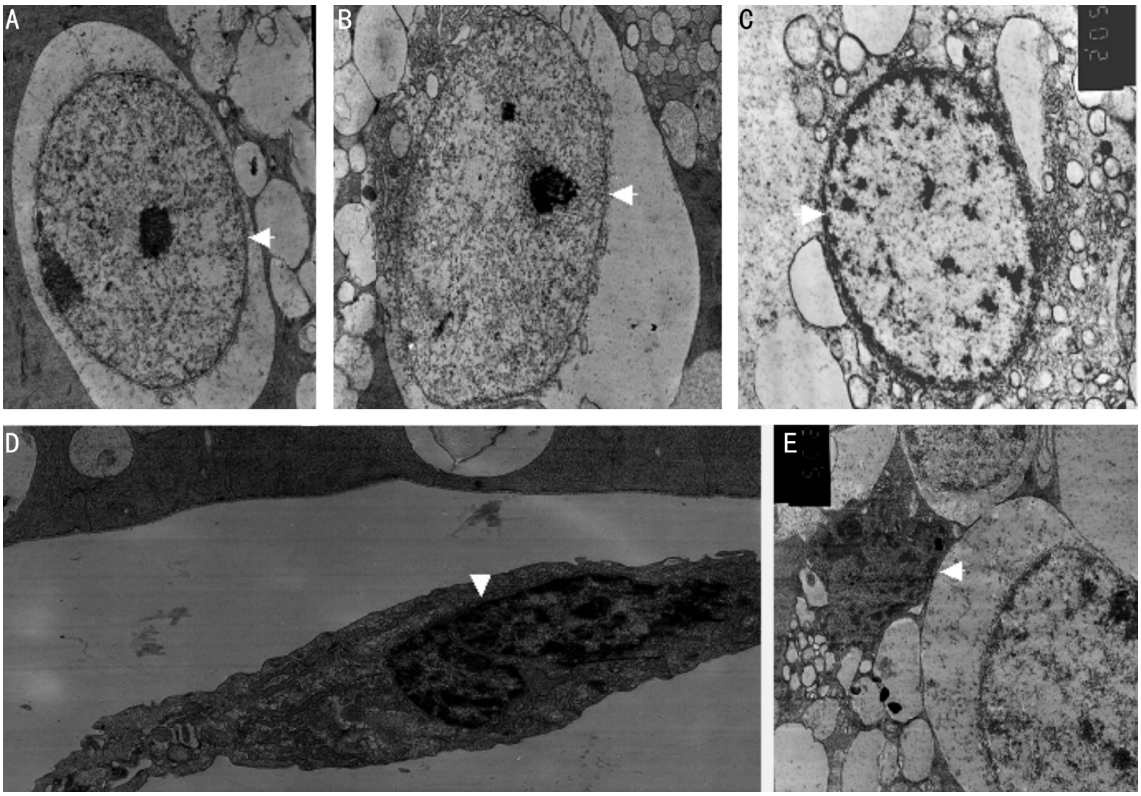

Figure 1 Electron microscopic photomicrographs of ganglion cell layer that show the toxic effects of rifampicin A: Control group; B: 50μg/0.1mL group; C: 100μg/0.1mL group; D: 150μg/0.1mL group; E: 200μg/0.1mL group.

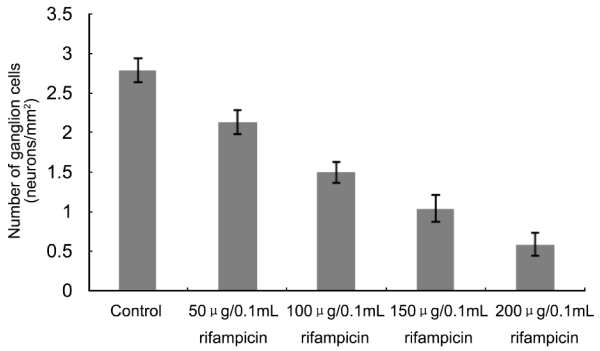

Figure 2 The number of ganglion cells in all groups.

DISCUSSION

Rifampicin is widely used as one of the first-line drugs in the treatment of TB. The common structure of rifampicin is a naphthohydroquinone or naphthoquinone chromophore spanned by an aliphatic ansa chain [10]. This lipophilic ansa chain is mainly responsible for the transport of the drug across the blood-brain/retinal barriers [11]. The structural feature of its naphthohydroquinone ring suggests that rifampicin may function as a hydroxyl radical scavenger, and it has been shown to inhibit β-amyloid (Aβ) aggregation and Aβ-induced neurotoxicity [12]. The neuroprotective actions of free radical scavengers in neurodegenerative disorders are well established[13].

Recent studies have shown that rifampicin binds to and activates glucocorticoid receptors (GRs), resulting in the induction of transcription of genes controlled by glucocorticoid receptor-binding elements [14]. GRs are expressed in isolated Müller and photoreceptor cells in intact salamander retinas and in all cell types in the eye [15]. Activated GRs can inhibit AP-1, which is essential for light-induced photoreceptor apoptosis by light. GR-mediated inhibition may occur in the nucleus of retinal cells by a protein-protein interaction of both transcription factors. Thus, induction of GR activity prevents light-induced retinal degeneration by interference with AP-1-dependent steps of apoptosis induction in mice [16]. Gollapudi *et al* [17] have reported that rifampicin-mediated inhibition of apoptosis and activation of caspase-3 and capase-8 occur at least in part *via* GR activation. Furthermore, rifampicin down-regulated the expression of pro-apoptotic Bax and up-regulated the expression of anti-apoptotic Bcl-2 and Bcl-XL and of anti-apoptotic gene products such as XIAP, cIAP2 and FLIPs, which play essential roles in blocking programmed cell death. Rifampicin is a well-established and generally well-tolerated drug for the treatment of TB.

Recently, rifampicin has been reported to induce neurodegeneration in the optic nerve transection model. The visual loss induced by rifampicin toxicity is reported to be mediated through an excitotoxic pathway whereby the drug disturbs the mitochondrial function; its toxicity depends on decreased ATPase activity and mitochondrial homeostasis[18]. Because of its lipophilic properties, it can easily enter the intracellular fluid as well as all body fluids and tissues. The optic neuritis is abrupt in onset and is generally seen at 3-6

months of the onset of treatment[19,20].

Many texts suggest that toxicity due to rifampicin is generally preventable with appropriate dosing, screening and careful monitoring and that if toxicity occurs, it is usually reversible. Rifampicin causes visual acuity, which appear to be due to optic neuritis. This effect may be related to dose and duration of treatment. This effect is generally reversible when administration of the drug is discontinued promptly[21].

In the present study, we evaluated the neurotoxic effects of rifampicin on retinal ganglion cells. After rifampicin administration, we evaluated the number of neuron cells. We saw that when the 50mL/g rifampicin dose was applied, the number of ganglion cells was nearly the same with the control groups' cell number. However, the most important factor is about the rifampicin doses. When the dose was increased, the cell numbers in the eye's retinal ganglion cell layer decreased gradually (Figure 2). Additionally when the rifampicin doses were increased again, the number of cells in the eye's retinal ganglion cell layer decreased. Moreover, when the 200mL/g rifampicin dose was applied, the number of ganglion cells decreased more than 5 times when compared with the control group. Therefore, low-dose rifampicin may be safe for intravitreal treatment of ocular TB.

Rifampicin toxicity is dose-related and is rare when the dose does not exceed 50mg/kg. Optic nerve toxicity with rifampicin use appears to be unpredictable, and the drug should be used cautiously.

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