GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(Research Article)

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The citicoline effect of choroidal and scleral thickness in *Rattus norvegicus* myopia model: A preliminary study

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GSC. Biological and Pharmaceutical Sciences, 2024, 28(01), 262–267

Publication history: Received on 17 June 2024; revised on 27 July 2024; accepted on 30 July 2024

Article DOI: https://doi.org/10.30574/gscbps.2024.28.1.0279

Abstract

This preliminary study aim to analyze the effect of citicoline to the scleral and choroidal thickness in rattus norvegicus myopia model. This study used lens induced myopia (LIM) method, by giving -10.00 D lens that framed with rubber ring for 3 weeks. The using of ruber as a lens frame has more complication risk than silicone frame. The 300 mg/kgBW citicoline treatment group increase the posterior segment od scleral and choroidal thickness. It could be concluded that citicoline could change the average scleral and choroidal thickness in rattus novergicus myopia model.

Keywords: citicoline; Choroid; Lens induced Myopia method; Myopia; Sclera

1. Introduction

Myopia is the most common cause of refractive error especially in children and young adult. The incidence of myopia is predicted increase sharply, about 2.5 billion at the end of decade. In 2000, an estimated 1.4 billion people were myopic, with predictions that this number will reach 4.8 billion by 2050 (1–3). Both genetic and environment influence the incidence of myopia. The myopia onset in children became earlier during pandemic because of most spending significant time activity during pandemic is near working like reading and using computers and smart phones (4–6). Many studies describe that near activity in children can influence the axial length of the eyeball that is the most common caused factor of myopia. Socioeconomically, uncorrected refractive errors can affect school performance, limit employability, and impair quality of life. Myopia is caused by the longer axial length of the eyeball. The longer axial length of the eyeball in myopia also associated with several eye complications, including retinal detachment, glaucoma, cataracts, optic disk changes, and maculopathy (7–10).

There are many research previously study about its pathogenesis and management on purpose control the progressivity of myopia. Numerous studies focus on controlling myopia by targeting the sclera. The sclera, the outermost layer of the eyeball wall, it determines the shape and length of the eyeball. The exact biomolecular pathogenesis of myopia in the sclera remains uncertain (6,11,12). Sclera is made up of fibroblast cells and extracellular tissue. In cases of severe myopia, the sclera becomes thinner especially at the posterior segment of the eyeball. Choroid, the vascular tissue is one of the layers of the eyeball that also have important rule in myopia, because choroid transfer the changes of the retinal neuromodulator effect to sclera that finally affect to the scleral biomolecular and biomechanic that influence the scleral thickness and its strength to maintain the shape of the eyeball (13–15). Citicoline, is part of the dopaminergic pathway, may serve as potential agents for controlling the progression of myopia. Citicoline is known for its positive effects in treating glaucoma and amblyopia, though research on its impact on myopia is limited. Citicoline (CDP-choline) can activate the biosynthesis of structural phospholipids in neuronal membranes and stimulate various neurotransmitter systems, including the dopaminergic system. Once released, the neurotransmitter diffuses laterally to the retina and choroid (16,17).

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The aim of this study is as a preliminary study to evaluate the effectiveness of the LIM method in creating a myopia model and to assess the impact of citicoline on changes in axial length, scleral thickness, and choroid thickness induced by the LIM method.

2. Material and Methods

2.1. Animal Model

The animal model testing in this study adhered to ethical criteria set by the Bioscience Institute of Brawijaya University and received ethical approval under certificate No. 049-KEP-UB-2024 from the Bioscience Institute of Brawijaya University, Malang, Indonesia. The study used the right eye of male Rattus norvegicus, aged 4 weeks and weighing 150–200 g. The rats were divided into a control negative group (n=2) , control positive group (n=2), and treatments group (n=2).

2.2. Myopia Induced Method

This study the myopia was induced using lens induced myopia (LIM) method. Prior to lens installation, anesthesia was administered via peritoneal injection of 0.03 ml ketamine and 0.02 ml xylazine during the suturing of a CR 39 multicoat lens with a power of S-10.00 D, diameter of 10 mm, and a 14 mm rubber frame around the rat's periorbital right eye. The LIM procedure lasted for 3 weeks. At the end of the treatment period, the rat was euthanized, and the right eye was removed for analysis. Rats that developed infections or ocular inflammation due to periorbital suturing were excluded from the study.

2.3. Citicoline treatment

Male Wistar rats (n= 10) were divided into five groups, there are (a) Negative control group (n = 2): No lens or treatment. (b) Positive control group (n = 2): An S -10.00 D lens was attached but not treated with citicoline. (c) Treatment group 1 (n = 2): An S -10.00 D lens + 200 mg/kg bw/day of citicoline. (d) Treatment group 2 (n = 2): An S -10.00 D lens + 300 mg/kg bw/day of citicoline. (e) Treatment group 3 (n = 5): An S -10.00 D lens + 400 mg/kg bw/day of citicoline.

2.4. Scleral thickness and choroidal thickness measurement

The eyeballs were enucleated and keep it to the Hartmann solution for 24 hours before moving to formalin solution. The eyeballs were cut in sagittal plane and stained with hematoxylin eosin. The sclera and choroid were measured at anterior, equator and posterior part using light microscope and image J application in μm. This study present the data on scleral and choroidal thickness using the average of each group that there are 2 subjects in each group.

3. Results and discussion

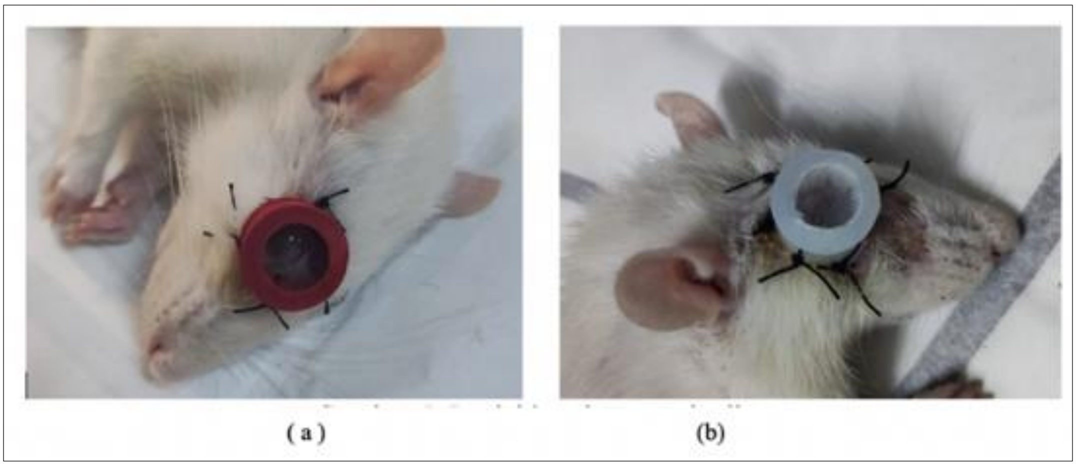

Figure 1 The Lens Induced Myopia (LIM) Method This study we used 10.00D lens that sutured at rat's palpebra with for 3 weeks. First we framed the lens with red rubber, it was too hard to be sutured at the rat's palpebra and easily came out and more caused palpebral injury (a). We replaced the red rubber frame into white silicone, it is easier to be sutured and less likely to caused injury (b)

The animals were monitored every two days. Figure 1 shows during the study, several lenses with rubber frame were more likely to come off due to rat's injured skin, compared to frames made of silicone so we change the red rubber frame with silicone and increased the location of stitching from four to six locations.

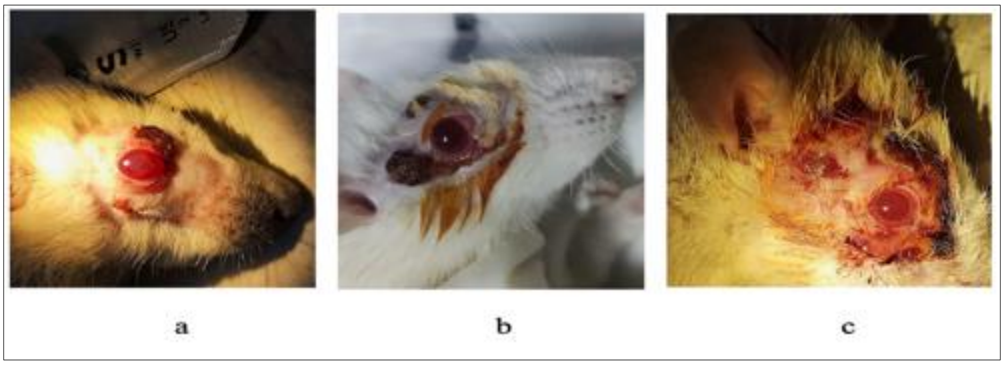

Figure 2 The complication during LIM methods. The 3 rd day, the red rubber frame injured the rat's palpebra and we encountered complications. We found one rat developed a corneal ulcer, there is an infiltrate in the cornea (a), one rat experiences a hematoma at the inferior palpebra, it possibility caused by the needle when we sutured the frame on to palpebra and one rat had apalpebral abscess, it possibility caused by the frame fricition(c)

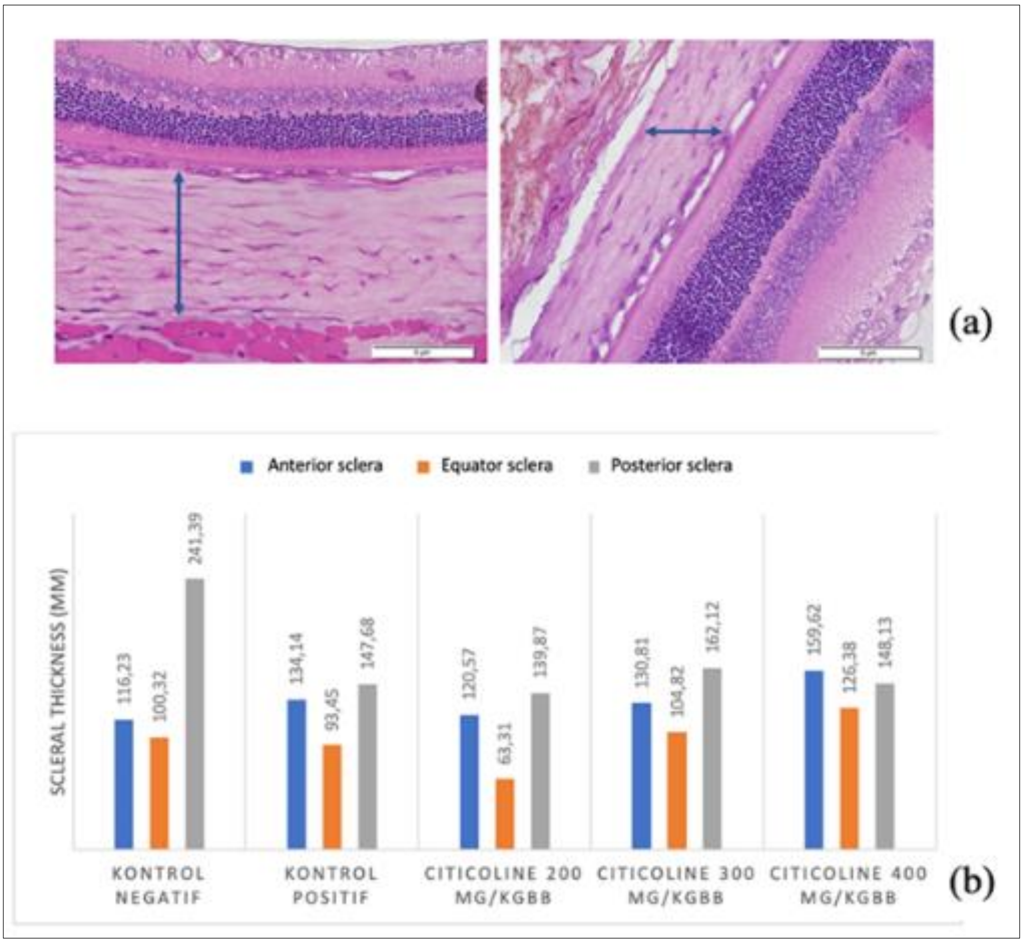

Figure 3 The *Rattus norvegicus* scleral thickness average, the measurement of scleral thickness was performed at the 3rd week of LIM method. The eyeball of the mouse was cut in a sagittal plane, it was made a preparation with hematoxylin eosin staining. The thickness of the sclera was then measured, the arrows indicating the thickness of the sclera(a). The graphic describe that the negative control group has the thickest posterior scleral thickness while among the citicoline treatment groups, the 300 mg/kg bw group has the thickest posterior scleral thickness (B)

Figure 2 describes that there were three rats suffering from complication, one rat got corneal ulcer, one rat got palpebral hematoma and one rat got palpebral abscess. We exclude the rats and we change tall lens frame with silicone ring.

After inducing myopia using the LIM method for 3 weeks, the rats were sacrificed, and enucleation was performed and they were stored in Hartmann's solution for 24 hours before they were moved into formalin. Figure 3 describes of the measurement of the *Rattus norvegicus* scleral thickness after 3 weeks LIM and citicoline treatment. A sagittal section was made for slide preparation and stained with hematoxylin eosin (HE), then the thickness of the sclera and choroid was measured using light microscope and image J application in μm.

The negative control group has the thickest of the scleral posterior segment (mean 241.39μm), while the citicoline 300 mg/kg bw treatment group has the thicker posterior scleral segment (mean 162.12 μm) than the positive control group. This condition is same with most of the literatures, most of them describes that, there is a changes in scleral structure that it decrease the number of collagen especially at posterior scleral segment so the posterior scleral segment become tinner in myopia. The changes of the posterior segment of the sclera affects the scleral elasticity and increase the creep rate of the axial length that makes the axial length of the eyeball become longer and increase the risk of myopia (13,18).

The choroidal thickness in this preliminary study was measured at anterior, equator and posterior part of the eyeball. Figure 4 show that, there is a differences of choroidal thickness in negative control, positive control and various dose of citicoline treatment group. Negative control group has the thinnest posterior choroid, while in the 300 mg /kg bw citicoline group has the thickest posterior choroid (24.16 μm) among other treatment groups.

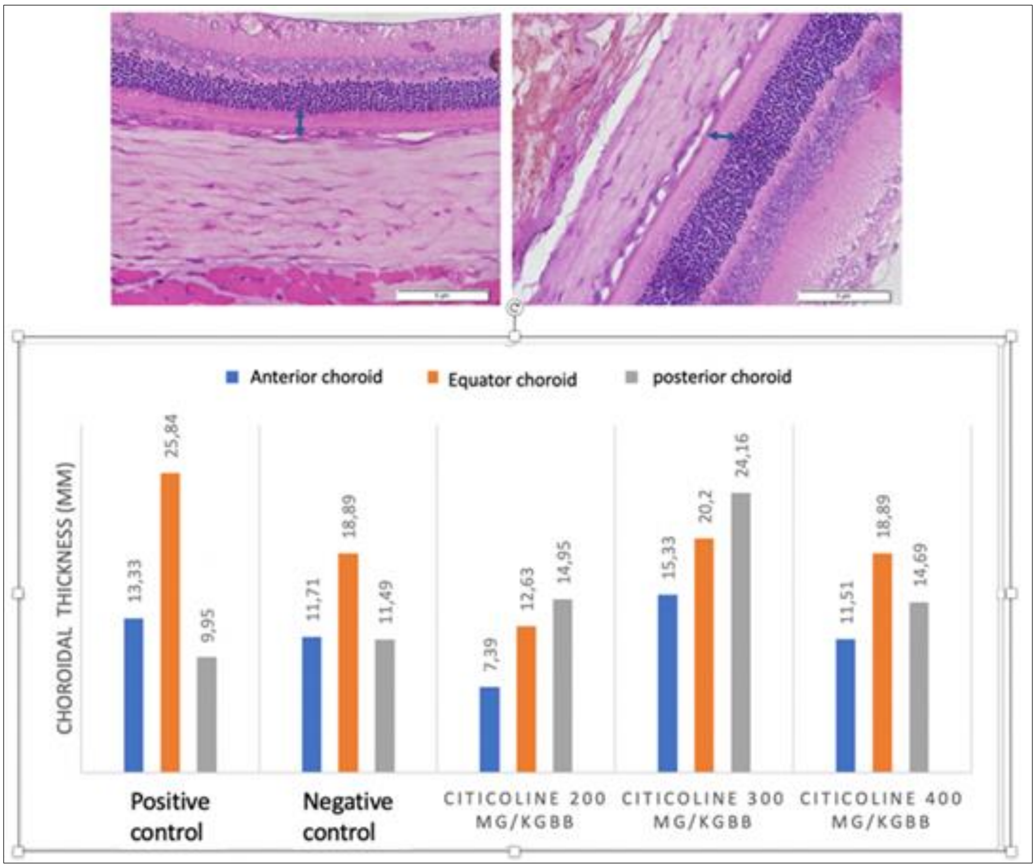

Figure 4 The *Rattus norvegicus* Choroidal Thickness Average. The Measurements of choroidal thickness was performed at the 3rd week of LIM method. The eyeball of the mouse was cut in a sagittal plane, it was made a preparation with hematoxylin and eosin staining. The thickness of the sclera was then measured, the arrows indicating the thickness of the sclera(a). The graphic describes that the negative control group has the thinnest posterior choroidal thickness while among the citicoline treatment groups, the 300 mg/kg bw group has the thickest posterior scleral thickness (B)

This is very interesting, the possibility of thickening of the choroid in the citicoline therapy group is likely due to compensation of the citicoline effect on the LIM treatment. However, this requires further research. Based on the literatures, choroid is vascular tissue that one of the eyeball layers also has an important role in maintain the scleral strength that influence the length and shape of the eyeball (19–21). By inducing myopia with LIM method it mean interfere the retinal amacrine cells neuromodulator. The changes of retinal neuromodulator composition affect to the choroid metabolism that changes the choroidal thickness (22–24).

The limitations of this study include the small number of animal models, which means the measurement results can only be averaged, and the suboptimal condition of the room and sterilization facilities, which increases the likelihood of infection or complications.

4. Conclusion

The Lens Induced Myopia (LIM) method during 3 weeks can change the scleral and choroidal thickness, so it could be predicted that this method could induced myopia in animal model and the 300 mg/kg bw citicoline treatment group can increase the sclera and choroid thickness compared with the positive control group. The choose of silicone frame for the lens decrease the risk of complication.

Compliance with ethical standards

Acknowledgments

this study is supported by research and community service unit of Faculty of Medicine Universitas Brawijaya , Indonesia

Disclosure of conflict of interest

There is no conflict of interests among of all researchers in this study.

Statement of ethical approval

The ethical clearance was declared by ethical clearance commission of Universitas Brawijaya number 049-KEP-UB-2024

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