Experimental Studies

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Retinal morphological changes in non-infectious uveitis in rabbits experimentally treated with citicoline versus non-treated rabbits

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Purpose: *To identify retinal morphological changes in a model of non-infectious anterior and intermediate uveitis and to assess the efficacy of experimental neuroprotective citicoline therapy in the treatment of the disease.*

Material and Methods: *Forty rabbits were divided in two experimental groups, the non-treatment group (18 non-treated rabbits) and treatment group (22 rabbits treated with the neuroprotector). A horse serum rabbit model was used for inducing uveitis. Histological structure of the retina was assessed on days 33 to 54 after the onset of uveitis.*

Results: *In the non-treatment group, there were retinal areas exhibiting marked destructive changes (sites of edema and disorganization in the inner nuclear layer, tractions between the retina and vitreous, reduced numbers of neuronal layers in the nuclear layers, and reduced numbers of ganglion cells) as well as areas of relatively well-preserved retina. At late time points after the onset of uveitis, animals treated with citicoline exhibited an almost normal retinal structure.*

Conclusion: *Horse serum-induced non-infectious anterior and intermediate uveitis contributed to retinal neurodegenerative changes, but uveitic animals treated with the neuroprotector for 33-54 days exhibited only minor neurodegenerative changes.*

Keywords:

retina, non-infectious uveitis, neurodegeneration, neuroprotection, uveitis model, pathogenesis

Non-infectious anterior and intermediate uveitis is the most common group of uveitis, affects mostly young individuals, results in a significant reduction in visual acuity, and is a common cause of visual disability [1-3].

Macular edema and optic nerve edema are serious complications of uveitis which affect visual acuity and may result in visual disability. The causes of these complications, however, have been not completely elucidated [4]. Studies have shown variability in the percentage of these complications [5, 6].

Morphological changes in the retina and optic nerve in experimental uveoretinitis have been described and may be attributed to the degenerative effect of substances used for inducing uveitis [7-9]. There is, however, lack of data for forming a complete picture of pathological changes in the retina.

General anti-inflammatory therapy, local and systemic glucocorticosteroids, immunos-uppressants, and biological therapy have been used in the treatment of non-infectious anterior and intermediate uveitis, but there is lack of data on the effects of neuroprotective medications in the treatment of this disease [2, 3, 10, 11, 12].

Citicoline is one of the best well-known and studied neuroprotectors [13]. In ophthalmology, it has been used in optic nerve lesions of various etiology, first and foremost, glaucomatous [14, 15, 16]. To the best of our knowledge, there have been, however, no reports on the use of citicoline in the treatment of non-infectious anterior and intermediate uveitis.

The purpose of this study was to identify retinal morphological changes in a model of non-infectious anterior and intermediate uveitis and to assess the efficacy of experimental neuroprotective citicoline therapy in the treatment of the disease.

Material and Methods

Forty Chinchilla rabbits (weight, 2.5-3.0 kg) were included in this study. They were housed and maintained under conventional vivarium conditions.

They were divided in two experimental groups, the control group (18 rabbits; 18 eyes) and treatment group (22 rabbits; 22 eyes). Non-infectious uveitis was induced in both groups, and the latter group, but not the former group, was treated with experimental citicoline (Farmak JSC, Kyiv, Ukraine) therapy in the presence of the induced disease.

Twenty rabbits (9 rabbits in the control group and 11 rabbits in the treatment group) were euthanized at days 8 to 13 after inducing uveitis following the disappearance of the symptoms of active uveitis (tearing, photophobia, injection, precipitates, fresh synechiae and fibrin in the anterior chamber, dilated iris vessels, iris edema and evidence of anterior chamber cells. In addition, another 20 rabbits (9 rabbits in the control group and 11 rabbits in the treatment group) were euthanized at days 33 to 54 after inducing uveitis, following the normalization of the temperature in the projection of the ciliary body which indicated the disappearance of inflammation [17]. Histological specimens obtained from both groups were examined by light microscopy.

Non-infectious anterior and intermediate uveitis was induced by the methodology described by Dorokhova and colleagues [18]. Animal body was first sensibilized by injecting normal sterile horse serum (BIOWEST SAS, Nuaille, France) at a dose of 1.0 ml daily for 5 days. A provoking dose of 0.1 mL of normal sterile horse serum was injected intravitreally into the right eye, whereas the left eye remained intact, in 10 days. A day thereafter, inflammation (anterior and intermediate uveitis) developed in the uvea.

Rabbits in the treatment group were receiving daily 0.2-ml citicoline administered intramuscularly throughout the observation period (from the first day of the onset of inflammatory process). Ophthalmoscopy and biomicroscopy were employed to monitor the clinical course of uveitis in animals in both groups.

For histological grading, we used the inflammatory score proposed by Dorokhova and colleagues [19]. Each variable characterizing the clinical picture was assigned 0 to 2 points based on symptom severity. Such important clinical signs of a severe uveitis course as the presence of fibrin in the anterior chamber and uveitis-associated failure to visualize the vitreous were assigned a score of 2. Such signs as tearing, photophobia, injection, corneal edema, precipitates, iris edema, dilated iris vessels, cell suspension in the anterior chamber, hypopyon, and fresh synechae were assigned a score of 1 [19].

Rabbits were sacrificed by air embolism after intravenous overdose using sodium thiopental (50 mg/kg). After animals were euthanized, enucleated eyes were fixated in 10% formalin, embedded in paraffin, cut into 5-μm sections, stained with hematoxylin and eosin, and sent for histomorphological examination using light microscopy. Histomorphological studies were conducted at the Pathology and Electronic Microscopy Laboratory of SI "The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine". Images were captured at various magnifications using a PowerShot A480 camera (Canon Inc., Tokyo, Japan) attached to a Laboval 4 light microscope (Carl Zeiss, Jena, Germany).

Statistical methods were not applied.

All animal experiments were performed in compliance with the Law of Ukraine on Protection of Animals from Cruel Treatment No. 3447-IV dated 21.02.2006 and European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes from the European Treaty Series (Strasbourg, 1986). The study was approved by the local Bioethics Committee of SI "The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine" (meeting minutes dated October 11, 2021) and conducted within the framework of the 2019-2020 research project "Investigating the neuroprotective effect of pyrimidine nucleotides on retinal ganglion cells and optic axons in endogenous anterior uveitis" (state register number, 0119U101224).

Results

On day 1 of uveitis, the inflammation score ranged 7 to 12, and on day 5, from 9 to 15. Thereafter, inflammation gradually subsided, and its signs were completely absent on days 8 to 13. No ophthalmoscopic evidence of inflammation was observed on days 33 to 54.

We found that, in control animals with induced non-infectious anterior and intermediate uveitis, the retinal structure appeared generally maintained and the retinal layers and individual cells within them could be distinguished. On days 8 to 13 of uveitis, we observed penetration of inflammatory vitreous lymphocytes into the retinal tissue (e.g., the retinal internal nuclear layer (INL)) (Fig. 1).

Along with sites of minor changes (Fig. 2), there were retinal sites with signs of dystrophic and degenerative changes. There were also sites of edema and disorganization in the retinal INL, and, consequently, no separation between the retinal INL and outer nerve layer (ONL) was evident. Some retinal locations exhibited marked destructive changes with loss of structure in photoreceptors (Fig. 3).

On days 33 to 54 after the onset of uveitis, progressive retinal destruction was observed in the samples from control (non-treated) animals. This was indicated by a number of pathological changes, including those which had not been observed at earlier time points (appearance of tractions between the retina and vitreous, reduced numbers of neuronal layers in the nuclear layers, and reduced numbers of retinal ganglion cells) (Fig. 4).

Therefore, control rabbits (i.e., those not treated a neuroprotective medication) exhibited progressive retinal degeneration and destruction at late time points. Nevertheless, some retinal locations exhibited an almost normal structure.

Uveitic animals treated with the neuroprotector citicoline exhibited varied numbers of the cells infiltrating the retina and vitreous at various retinal and vitreous locations, with some of the locations showing no immune protective cells. The retinal structure appeared almost unaltered, with well seen retinal layers (the retinal photoreceptor layer, outer and inner nuclear layers, outer

and inner ganglion layers and nerve fiber layers) and with no locations showing loss of the retinal layered structure (Fig. 5). There were isolated retinal ganglion cells showing dystrophic changes (Fig. 6).

At late time points, uveitic animals treated with the neuroprotector citicoline exhibited an almost normal retinal structure and necrotic immunocompetent cell debris with no nucleus in the vitreous (Fig. 7).

Therefore, the rabbits not treated with citicoline for their anterior and intermediate uveitis showed large numbers of various immunocompetent cells resulting in ocular trophic and metabolic abnormalities, with the latter leading to neuronal and ganglion cell death and loss of the layered structure.

Marked inhibition of dystrophic and neurodegenerative processes in the retina at weeks 1 and 2 and day 55 after the onset of uveitis was observed in animals treated with citicoline compared to intact controls with non-infectious uveitis.

Discussion

This experimental study found that uveal inflammation is accompanied by neurodegenerative changes in the retina. Our findings are in line with those of a mouse model study of uveoretinitis. Experimental autoimmune uveoretinitis was induced in the B10.RIII mice following immunization with bovine interphotoreceptor retinoid binding protein (IRBP) and human IRBP161–180 peptide. Disease was typically of an acute nature, characterized by rapid onset of a massive inflammatory response, resulting in extensive damage to the rod outer segments (ROS) and neuronal layers [20, 21]. Forrester and colleagues [22] studied the morphology of S-antigen-induced uveoretinitis in guinea pigs. Purified bovine retinal S-antigen was shown to produce a focal chorioretinitis, characterized by selective damage to the outer retina. Because uveoretinitis was induced using the substances that cause the immune response of the retina, it is understandable that degenerative changes did develop in the retina. As opposed to the above studies, for the purpose of immunization, we used horse serum that has no direct immunogenic effect on the retina and its components. It is for this reason that we consider our finding of the degenerative changes in the retina and optic nerve a complication of anterior and intermediate uveitis, but not that resulting from the direct effect on the retina.

Our study demonstrated that retinal dystrophic and degenerative changes develop in the animal model of non-infectious anterior and intermediate uveitis. We found the altered histological structure of the retina, retinal infiltration with polymorphic cells, and regions of degenerative changes with dissociation of the layers, swelling and neuronal loss. Our findings justify the need for the use of neuroprotective medications for preventing or reducing retinal damage in anterior and intermediate uveitis.

We believe that citicoline is promising for preventing retinal damage in anterior and intermediate uveitis. The medication was found to be effective in rat models of methanol- intoxicated retina [23] and ethambutol opric neuropathy [24] and in patients with disseminated chorioretinitis and optic neuritis [25].

It has been hypothesized that the mechanisms of retinal protection by citicoline may involve suppression of ganglion layer edema, increase in antiapoptotic protein expression, and decrease in proapoptotic protein expression [23] with the involvement of several metabolic pathways. In addition, the rationale for the use of citicoline in ophthalmological neurodegenerative diseases is founded on its multifactorial mechanism of action and the involvement in several metabolic pathways, including phospholipid homeostasis, mitochondrial dynamics, as well as cholinergic and dopaminergic transmission [26]. Anti-oxidant and anti-inflammatory properties of citicoline are also important [27].

A key protective effect of the medication appears to be associated with its capacity to reduce retinal edema [23]. Pichi and colleagues [28] used optical coherence tomography (OCT) to analyze vitreous anatomy of anterior uveitic patients. They concluded that OCT evidence of a connecting channel between the premacular bursa and the Cloquet's canal suggests that inflammatory cytokines may drain from the anterior chamber through this system of channels, thus increasing the risk of cystic macular edema. Edema severity may depend on the activity of the inflammatory process in uveitis [28]. Retinal edema, in turn, substantially affects retinal plasticity, with critical retinal plasticity values leading to irreversible retinal deformations [30]. The appearance of regions of retinal disorganization and destruction in experimental uveitis in the current study may be explained by the above mechanism.

This study demonstrated that experimental citicoline therapy resulted in a reduction in retinal damage in early and late non-infectious anterior and intermediate uveitis in rabbits. Thus, the retinal structure was almost normal, with only minor residual changes, at late time points of observation. Therefore, it could be believed that the neuroprotective medication improved the state of the rabbit retina that had been under the very unfavorable uveitic conditions for almost two months. Naturally, citicoline could not completely arrest the retinal degenerative processes initiated by intraocular inflammation, but it hampered the progression of these processes and, therefore, hampered their transition to neurodegeneration.

Conclusion

Horse serum-induced non-infectious anterior and intermediate uveitis contributes to progressive retinal neurodegeneration with neuronal and glial loss and an altered histological structure of the retina. The use of the neuroprotectot citicoline prevents alterations in the histological structure of the retina and ameliorates degenerative changes in retinal cells at early (days 8-13) and late (days 53-54) time points of experimental non-infectious anterior and intermediate uveitis.

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Disclosures

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Conflict of interest: *All authors declare they have no financial interests.*

Author Contributions: *Concept and design of the study: OVZ, OED; data curation and data analysis: ISH, OVZ, OED; histopathological study: ISH, EVM; drafting and critical revision of the manuscript: ISH, OVZ, EVM, OED. All authors read and approved the final manuscript.*

Disclaimer: *The opinions presented in this article are those of the authors and do not necessary represent that of SI "The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine".*

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Data Availability Statement: *All the data obtained or analyzed during this study are reported in the article.*

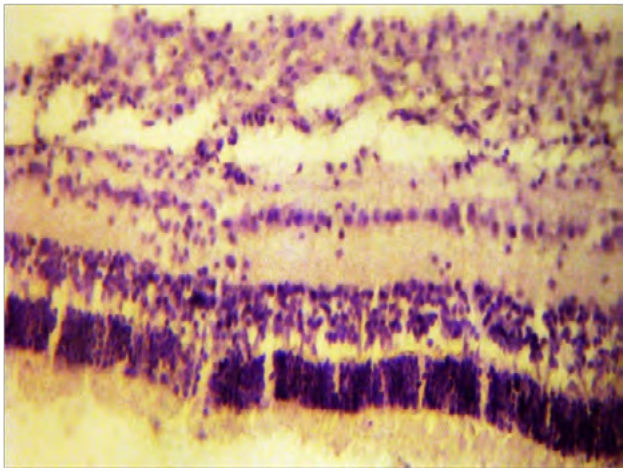

Fig. 1. Retinal histological section of a rabbit with non-infectious anterior and intermediate uveitis at day 11 after disease onset. Lymphocytic infiltration of the inner retina of the affected eye. Hematoxylin and eosin staining. Original magnification 160×.

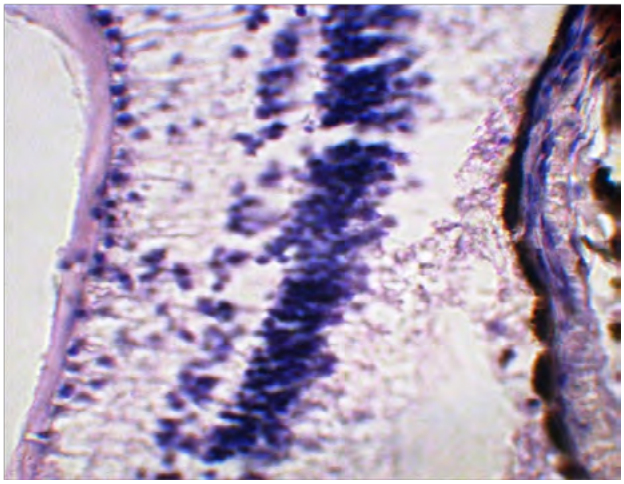

Fig. 2. Retinal histological section of a rabbit with non-infectious anterior and intermediate uveitis at day 11 after disease onset. Histological structure of the retina appears generally well preserved. Note isolated lymphocytes deep inside the retina. Hematoxylin and eosin staining. Original magnification 280×.

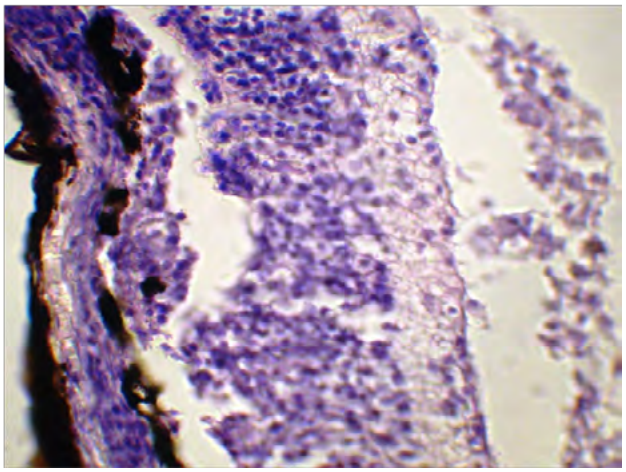

Fig. 3. Retinal histological section of a rabbit with non-infectious anterior and intermediate uveitis at day 11 after disease onset. Note destructive changes in the retina, a conglomerate of cells of the inner and outer nuclear layers, total loss of the photoreceptor layer and lymphocytic infiltration of the vitreous. The uvea appears expanded and infiltrated with inflammatory lymphocytes. Hematoxylin and eosin staining. Original magnification 280×.

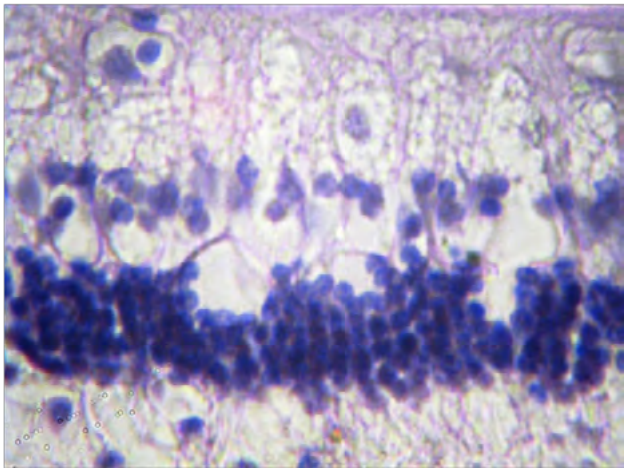

Fig. 4. Retinal histological section of a rabbit with non-infectious anterior and intermediate uveitis at day 50 after disease onset. Note a subatrophic retinal region with 1-2 neuronal layers in the inner nuclear later and 4-5 neuronal laters in the outer nuclear layer and a ganglion cell exhibiting destruction. Hematoxylin and eosin staining. Original magnification 640×.

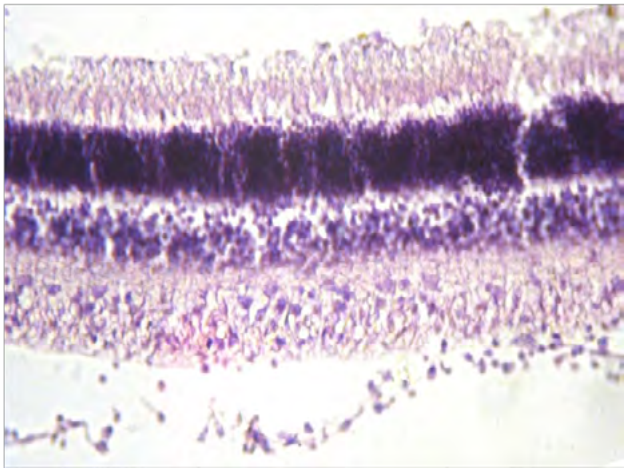

Fig. 5. Retinal histological section of a rabbit experimentally treated daily with citicoline for non-infectious anterior and intermediate uveitis; day 8 after disease onset. Note polymorphic cell infiltration of the vitreous and individual retinal regions. Hematoxylin and eosin staining. Original magnification 160×.

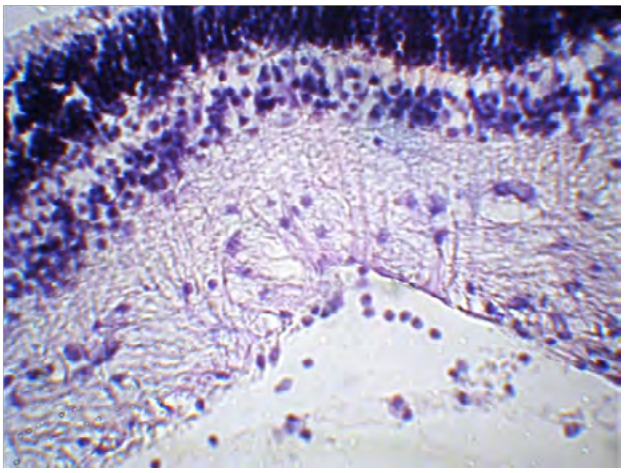

Fig. 6. Retinal histological section of a rabbit experimentally treated daily with citicoline for non-infectious anterior and intermediate uveitis; day 13 after disease onset. Note (a) a ganglion cell showing signs of vacuolized cytoplasm and (b) round-cell infiltration of the vitreous and individual retinal regions. Hematoxylin and eosin staining. Original magnification 280×.

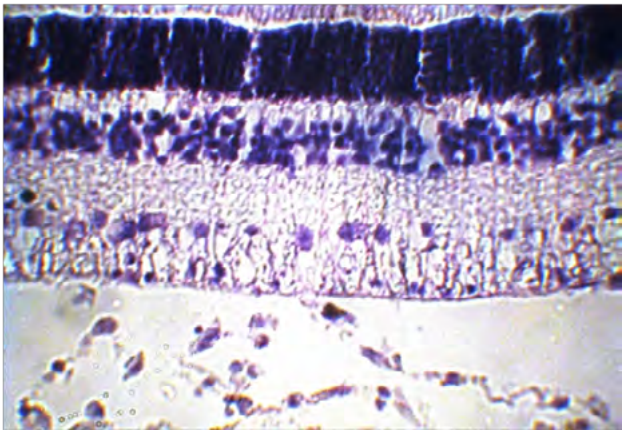

Fig. 7. Retinal histological section of a rabbit experimentally treated daily with citicoline for non-infectious anterior and intermediate uveitis; day 54 after disease onset. Note normal retinal structure and the vitreous exhibiting nucleus-less fragments of immunocompetent cells. Hematoxylin and eosin staining. Original magnification 280×.