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Late observation of the neuroprotective effect of citicoline in uveitis: an impact on the ultrastructure of the choriocapillaris, retina and optic nerve in rabbits

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Purpose: *To evaluate the ultrastructure of the choriocapillaris, retina and optic nerve (ON) in citicoline-treated versus non-treated rabbits at the late time point after inducing non-infectious anterior and intermediate uveitis.*
Methods: *The ultrastructure of the rabbit choriocapillaris, retina and ON was evaluated at day 55 after the initiation of uveitis.*
Results: *Experimentally induced uveitis caused neurodegenerative changes in the retina and ON. Neuroprotective treatment activated intracellular compensative processes resulted in reduced signs of hydropic degeneration and normalized cell ultrastructure, and contributed to the activation of metabolism in ON glial cells and axoplasm.*
Conclusion: *Neuroprotective treatment for non-infectious anterior and intermediate uveitis resulted in a reduction in neurodegenerative changes in the retina and ON.*

Introduction

Uveitis is a leading cause of visual impairment and responsible for about 20% of legal blindness [1]. Human leukocyte antigen (HLA)-B27 acute anterior uveitis is the most frequently recognized type of acute anterior uveitis and anterior uveitis overall [2, 3]. In 45% of cases, it is not, however, possible to establish a particular cause of ocular inflammation, which is considered idiopathic immune process [4] or non-infectious uveitis. The exact pathogenesis of non-infectious uveitis is, however, still unclear [5].

Although inflammation is responsible for defending the body from various injuries and plays an important role in tissue repair and regeneration, excessive inflammation can cause tissue damage and disease. In many cases, acute uveitis can become sluggish and chronic [6], with the involvement of the optic nerve (ON) and macular region of the retina into the pathological process and potential development of complications like optic disc edema and macular edema [7, 8]. If left untreated, chronic macular edema can lead to photoreceptor damage and irreversible vision loss. Moreover, macular edema can be observed even in the setting of well-controlled uveitis or secondary to previous inflammation and irreversible blood-retina barrier breakdown [9].

At present, corticoids form the mainstay of medical therapy for uveitis, which is aimed at reducing inflammation and pain [10, 11].

Neuroptotective therapy has become widely used recently and is believed to be promising because it is based on a pathogenetic approach. Citicoline is the international non-proprietary name of the substance whose chemical structure is cytidine-5′-diphospho-choline (CDPCholine). Endogeneously synthesized CDPCholine is naturally present in all living cells, where it serves as an obligatory intermediate in the synthesis of phosphatidylcholine (PtdCho), a major component of neuronal and mitochondrial membranes [12]. The molecule exhibits a neuromodulating action mainly in at the level of the dopaminergic system, which offers the rationale for citicoline use in treating glaucoma, dopamine being one of the main neurotransmitters involved in the visual signal transmission from the retina and optic disc to the visual cortex [13]. It exerts short- and long-term neuroprotective effects on retinal ganglion cells (RGCs) [14].

The purpose of the study was to evaluate the ultrastructure of the choriocapillaris, retina and optic disc in citicoline-treated versus non-treated rabbits at the late time point after inducing non-infectious anterior and intermediate uveitis.

Material and Methods

Four Chinchilla rabbits (4 eyes; 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 groups, the control group (2 rabbits; 2 eyes) and treatment group (2 rabbits; 2 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.

Uveitis was induced by the methodology described by Dorokhova and colleagues [15]. Uveal inflammation (non-infectious anterior and intermediate uveitis) developed one day after a provoking dose of 0.1 mL of normal sterile horse serum (BIOWEST SAS, Nuaille, France) was injected intravitreally into the right eye.

Thereafter, 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.

On day 55 after the development of uveitis, with the disappearance of inflammation indicated by the normalization of temperature in the projection of the ciliary body [16], rabbits in both groups were sacrificed by air embolism after intravenous overdose using sodium thiopental (50 mg/kg). The ultrastructure of the choriocapillaris, retina and optic disc was evaluated.

Tissue specimens were fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.4), postfixed in 1% osmium tetroxide (OsO4), dehydrated in a gradient series of ethanol until absolute, embedded in an Epon–Araldite mixture and polymerized. Thereafter, ultra-thin (500-700A°) sections were cut, stained with lead citrate according to the procedure described by Reynolds [17], and observed with a PEM-100-01 Transmission Electron Microscope (Selmi, Sumy, Ukraine). Images were captured at various magnifications using a PowerShot A480 camera (Canon Inc., Tokyo, Japan). Magnifications are specified in figure legends.

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 55 after the development of uveitis, choroidal choriocapillaris lumens appeared irregular, focally narrowed or expanded compared to controls. At some locations, lumens were significantly narrowed and blocked by hypertrophic endothelial cell (EC) bodies, and some EC appeared altered ultrastructurally. They appeared somewhat tortuously arranged and showed fenestrae and plasmallemal protrusions. Isolated leukocytes and platelets were seen in lumens. Signs of focal edema were observed in the ground substance of the connective tissue surrounding capillaries. Collagen fibrils were sparsely arranged, with active fibroblasts and isolated plasma cells and melanocytes seen in between them.

The retinal pigment epithelial (RPE) layer was arranged in a wave-shaped pattern. Isolated RPE cells with focal or total destruction of mitochondrial cristae and swollen inner mitochondrial matrix contained small numbers of melanosomes. The folds on the basal surface of membranes were either absent or shallow and short. Apical microvilli appeared small and rudimentary. Extracellular edema was observed in the RPE layer. Retinal layers below the RPE layer appeared completely filled with Müller cell processes and isolated altered RPE cells. Photoreceptor cells had no outer or inner segments. Some of residual photoreceptor nuclei appeared filled with condensed chromatin. Photoreceptor cells and other neural cells and their processes were occasionally seen between Müller cell processes and exhibited hydropic degeneration (Figs. 1, 2).

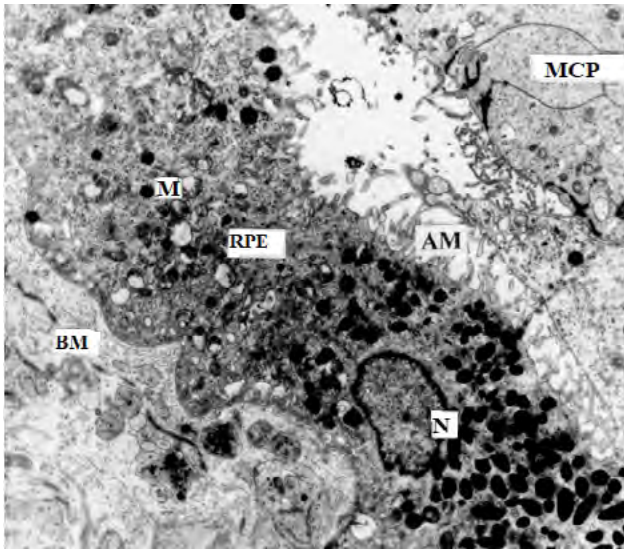

Fig. 1. Rabbit retina on day 55 after initiation of uveitis. RPE layer appears arranged in a wave-shaped pattern and pathological changes in RPE cells. Note the absence of photoreceptor cells and hyperplasia of Müller cell processes. Magnification, x4000. Note: AM, apical microvilli; BM, Bruch membrane; N, nucleus; M, mitochondria; MCP, Müller cell processes; RPE, retinal pigment epithelium

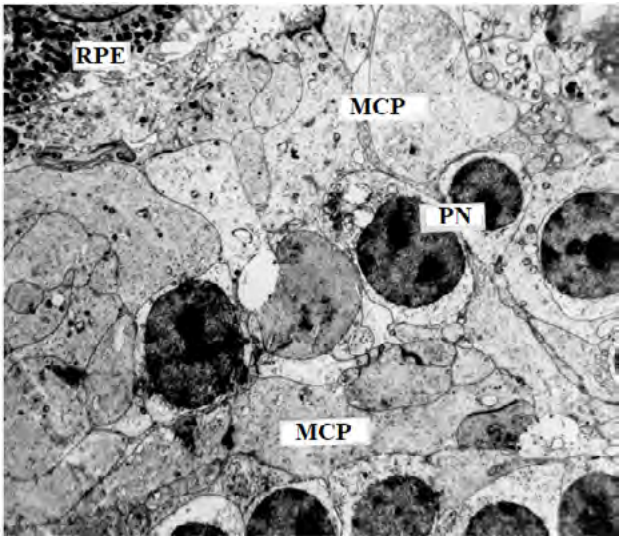

Fig. 2. Rabbit retina on day 55 after initiation of uveitis. Note hyperplasia of Müller cell processes, the absence of the inner and outer segments of photoreceptors and pathologic photoreceptor nuclei. Magnification, x2500. Note: MCP, Müller cell processes; PN, photoreceptor nuclei; RPE, retinal pigment epithelium

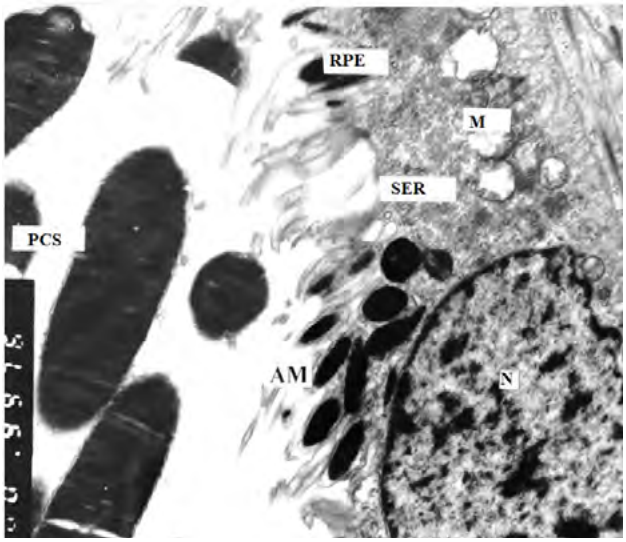

Fig. 3. Rabbit retina on day 55 of neuroprotective treatment for experimentally induced uveitis. Retinal pigment epithet;lial cell with signs of compensative-restorative processes. Magnification, x6000. Note: AM, apical microvilli; M, mitochondria; N, nucleus; POS, photoreceptor outer segments; RPE, retinal pigment epithelium; SER, smooth endopolasmic recticulum

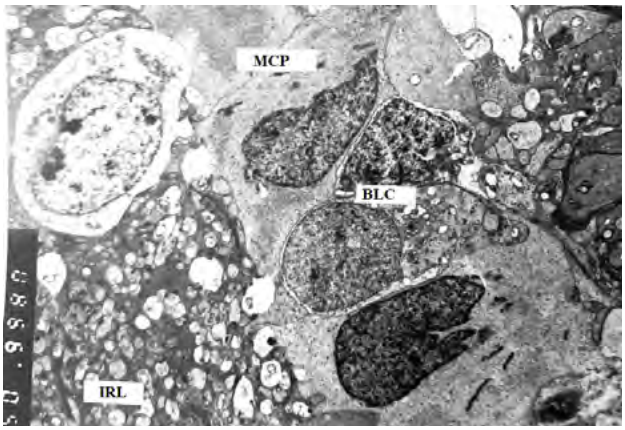

Fig. 4. Rabbit retina on day 55 of neuroprotective treatment for experimentally induced uveitis. Note hydropic changes in neural cells and their processes. Magnification, x2500. Note: MCP, Müller cell processes; BLC, bipolar layer cells; IRL, inner retinal layer

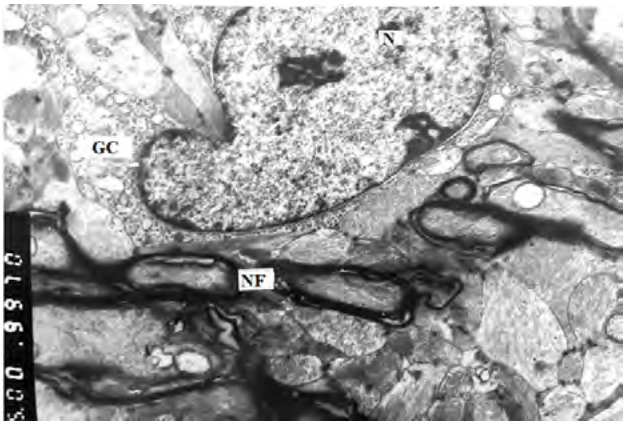

Fig. 5. Ultrastructure of the optic nerve on day 55 of neuroprotective treatment for experimentally induced uveitis. Note signs of reactive changes in mitochondria of nerve fiber axons and activation of glial cell metabolism. Magnification, x5000. Note: GC, glial cell; NF, nerve fibers

On day 55, some ON fibers from non-treated animals exhibited deformation of myelin sheaths, splitting of myelin lamellae, separation of the axolemma from the myelin sheath, and and axoplasmic swelling. Axoplasm showed mitocholdrial disorder with swollen inner mitochondrial matrix, almost total destruction of mitochondrial cristae and "fuzzy" microtubules and neurofilaments. Glial cells exhibited signs of hydropic degeneration. Some of these cells showed increased numbers of polysomes, which indicated increased protein-synthesis processes aimed at the restoration of intracellular structures. Signs of intercellular edema were occasionally seen in the ON.

After neuroprotective treatment of induced uveitis, the choriocapillaris exhibited active ECs with normal ultrastructure and signs of mild hydropic degeneration. Some choriocapillaris lumens appeared expanded with moderate electron density, showing aggregation of erythrocytes and isolated leukocytes.

The RPE layer appeared well developed, with its cells exhibiting polymorphic changes: some cells showed a normal ultrastructure with a typical set of organelles, whereas other exhibited alterations or signs of metabolic activation. The ultrastructure of photoreceptor outer and inner segments was normal, and photoreceptor cytoplasm exhibited increased numbers of polysomes. Mild hydropic changes were observed in isolated neural cells and their processes in other retinal layers (Figs. 3, 4).

On day 55, most ON fibers from citicoline-treated animals exhibited a restored myelin sheath thickness and shape and significantly reduced signs of axoplasmic and extracellular swelling. Some glial cells exhibited mild hydropic changes, whereas others exhibited signs of activated compensative-and-restorative processes (Fig. 5).

Discussion

We have previously found that, at early time points (days 8-13) after the initiation of experimental non-infectious anterior and intermediate uveitis, the inflammatory is accompanied by neurodegenerative changes in the retina, choroid and ON [18, 19]. There was light microscopic and ultrastructural evidence of impaired retinal cellular architecture and sites of destructive changes with disorganization of the retinal layers, edema, neuronal death, and polymorphonuclear infiltration [18, 19]. The retinal photoreceptor and pigment epithelial cells were most severely affected [18], but degenerative changes were observed also in the inner retinal layers, first and foremost, in RGCs [19]. Hydropic degeneration of endothelial cells of the choriocapillaris was typical for the choroidal abnormalities [18]. In experimental non-infectious uveitis, the ON exhibited destructive changes in neural fibers, with damage to the cytoskeleton of neuronal processes and myelin sheaths [18]. Of note that the presence of swelling was a common feature of damage to the ocular shells in the development of experimental anterior and intermediate non-infectious uveitis.

The current study found that, on day 55 after the initiation of experimental anterior and intermediate non-infectious uveitis, signs of swelling were still seen in the retina, choroid and ON, but there were also signs of reparative processes. It is noteworthy that the most apparent residual phenomena were seen in the retina and ON. In the retina, sites of damaged photoreceptor cells were replaced by glial Müller cells, and there were still apparent degenerative changes in RPE cells and swelling, which may impair the restoration of visual function after uveitis. Damage to the myelin sheath and axoplasmic swelling were still observed in the ON.

In total, our findings of the ultrastructural and optic microscopic evidence of changes in the ocular shells in early and late anterior and intermediate non-infectious uveitis indicate that swelling may be a key mechanism for retinal and ON damage, and relatively large damage to the retina and ON in anterior and intermediate non-infectious uveitis requires neuroprotective therapy.

With regards to tissue swelling, there are rheological models of the human retina which recreate the major features of non-monotonous plastic destruction in the presence of plastic deformation in the retina. These models allow evaluating the time required for retinal stretching (particularly that induced by swelling) to develop which would result in irreversible retinal destruction [20]. In the current study, swelling lasted for as much as 55 days after the initiation of uveitis. Therefore, swelling duration, first and foremost, in late uveitis, may be sufficient for the development or irreversible retinal deformation. In addition, a reduction in swelling may have a key value for the restoration of retinal functions in the later period of the disease.

Our previous studies confirmed the efficacy of citicoline in early non-infectious uveitis. The use of citicoline resulted in a reduction in photoreceptor damage, activation of intracellular restorative processes in the retina (particularly, the RPE) and reduction in myelin sheath deformation and axoplasmic swelling in ON fibers [18]. The prolongation of the neuroprotective therapy to day 55 after the initiation of the uveitis resulted in the minimization of retinal and ON damage. We believe that our findings indicate that the use of citicoline for preventing retinal and ON damage in experimental non-infectious anterior and intermediate uveitis is effective and targets pathogenetic mechanisms underlying the disease. It is the ability of the medication to reduce retinal edema may become a key efficiency feature of the medication [21].

The evidence of the ability to modulate RGC metabolism are other potential beneficial effects of citicoline which target pathogenetic mechanisms underlying the disease. Parisi and colleagues [22] reviewed all published papers on experimental or clinical studies about the effects of citicoline on RGC morphology. Experimental studies demonstrated that citicoline has the neuromodulateve effect and protective effect on RGCs.

Tezel [23] reported on changes in the ocular vasculature, connective tissue of the ON and mitochondria which may result in glaucomatous ON damage. He also

suggested that mitochondrial dysfunction, as is either a cause or consequence of damage, renders RGCs sensitive to degeneration. The changes similar to above were found in our study. Therefore, out findings confirm the efficacy and pathogenetic rationale for the use of citicoline for preventing retinal and ON damage in non-infectious anterior and intermediate uveitis.

Conclusion

On day 55 after the initiation of experimental non-infectious anterior and intermediate uveitis, we observed residual damage to choriocapillaris, retina and ON, and signs of activated restorative-and-compensative processes. Altered endothelial cells were still observed in the choriocapillaris and the retina exhibited signs of gliosis; the ON exhibited nerve fiber abnormality, intercellular swelling, focal structural loss and hydropic degeneration of glial cells. The use of the neuroprotector citicoline in non-infectious uveitis for 55 days activates intracellular compensative- and-restorative processes in the endothelial cells of the choriocapillaris, retina and ON, leading either to the restoration of their ultrastructure or a reduction in manifestations of cellular alterations.

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Disclosures

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All authors reviewed the results and approved the final version of the manuscript.

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