Experimental Studies

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Impact of a course of injections with melatonin on morphological and functional changes in the optic nerve in experimental animals with hypopinealism

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Background: *Optic atrophy (OA) may be expected in hypopinealism, which is accompanied by degenerative changes in the retina.*

Purpose: *To assess the impact of a course of injections with melatonin on the morphological and functional optic nerve (ON) changes in rabbits exposed to prolonged around-the-clock light (ATCL) leading to hypopinealism.*

Material and Methods: *Eighty-four rabbits were used in this experimental study. Group 1 (an ATCL group) was composed of 32 animals exposed to ATCL to develop functional hypopinealism. Group 2 (an ATCL+M group) was composed of 29 animals exposed to ATCL but treated with intramuscular melatonin for 14 days. Group 3 (a control group or CG) was composed of 23 intact animals maintained under natural day/night cycle conditions. Groups were subdivided into subgroups based on experimental constructs (1-2 months, 3-5 months, 8-12 months, 18-19 months, 26-28 months). Blood melatonin levels were assessed by commercially available enzyme-linked immunosorbent assay kits. ON specimens were obtained and comprehensively assessed morphologically and morphometrically.*

Results: *Night-time blood melatonin level in experimental groups was almost six-fold lower than that in controls. Signs of abnormal ON circulation were observed at ≤12 months of ATCL exposure. ON demyelination was observed from months 3-5 of the experiment. Sclerotic and atrophic processes in the ON were observed at 28 months of ATCL exposure. In ATCL26-28 and ATCL+M26-28 subgroups, the mean relative vascular area in the intraorbital ON was significantly reduced compared to CG26-28 (2.01 ± 0.15% and 1.93 ± 0.15%, respectively, versus 3.20 ± 0.13%, $p < 0.05$). In addition, the mean relative area of the perivascular connective tissue (4.80 ± 0.15% and 4.61 ± 0.17%, respectively) was significantly increased compared to CG26-28 (3.40 ± 0.14%, $p < 0.05$). Moreover, the mean diameter of the nerve fiber bundle (2.51 ± 0.09 ×10-6 m and 2.73±0.10×10-6 m, respectively) was significantly reduced compared to CG26-28 (3.85±0.14×10-6 m; $p < 0.05$).*

Conclusion: *The morphological findings (like demyelination of nerve fibers and thinning of nerve fiber bundles of the ON), combined with low blood flow in ON vessels, vascular wall thickening and connective tissue growth, indicated the development of sclerotic atrophy of the ON, in the presence of marked melatonin deficiency, in rabbits exposed to ATCL. The 14-day course melatonin treatment of ATCI-exposed rabbits exerted anti-edematous effects at early time points (< 5 months), until obviously irreversible changes in the ON occurred. However, the course melatonin treatment exerted no impact on the development of OA in animals with persistent, marked hypopinealism developed in the presence of prolonged (28-month) exposure to ATCI.*

Keywords:

optic nerve atrophy, around-the-clock light, hypopinealism, melatonin, morphological and functional changes

Introduction

Optic atrophy (OA) can result from pathological processes like degenerative abnormalities, compression of or injury to the optic nerve, optic nerve inflammation, edema, etc. Ascending OA is also known as Wallerian degeneration. This anterograde degeneration occurs as a consequence of injury to the retinal elements or optic nerve head axons. The subsequent degeneration ascends towards the lateral geniculate body and superior colliculus. Pathomorphologically, OA is manifested by a loss of smaller blood vessels, thinning and disintegration of neural fibers, and the variable amount of reactive gliosis and fibrosis [1, 2, 3]. Hypopinealism can cause OA and is accompanied by atherosclerotic and degenerative changes in the retina in the presence of melatonin deficiency [4].

The pineal gland accounts for about 80% of total melatonin circulation in humans or vertebrate animals. With the daily alternation of light and darkness, afferent fibers of the peripheral neural system convey neural impulses to the central nervous system (CNS) from the retina, thus enabling a rhythm of pineal gland melatonin production as well as the development of circadian rhythms [5–8].

Melatonin has been reported to be produced not only by the pineal gland, but also by enterochromaffin cells in the gastrointestinal tract [9], retinal photoreceptors [10, 11] and ciliary epithelial cells [12]. In both the pineal gland and ocular tissue, melatonin synthesis and release exhibit circadian rhythmicity, with highest levels in darkness, and lowest levels in the light [6, 8, 13–15].

Melatonin contributes to several physiological processes in the eye, including photoreceptor renewal in retina [16, 17], aqueous production and intraocular pressure modulation in the anterior chamber [18], wound healing on the ocular surface [19] and as an antioxidant in the crystalline lens [20]. Melatonin receptors are found throughout the ocular tissue, including the cornea, lens, ciliary body, retina, choroid and sclera [21].

In modern societies, exposure to artificial lighting substantially increases bright-light time during the day and, consequently, decreases the period of melatonin production in darkness, which greatly affects human biological rhythms, leading to chronic impairment in physiological processes. Even a short exposure to bright light at night can affect circadian rhythms. A persistent decrease in melatonin production can result in metabolic syndrome [22], leading to impaired cellular free radical activities and pathological changes in different tissues [23–25].

Experimental animal studies have reported that around-the-clock light (ATCL) exposure led to hypopinealism and significantly deficient melatonin production. They found that morphological and functional changes in the pineal and thyroid glands and marked dyslipidemia which underlies the pathogenesis of atherosclerotic coronary ischemic disease develop under these conditions [23, 25]. We have reported previously that retinal atherosclerotic and degenerative abnormalities [4], ciliary body vascular abnormalities and signs of gliosis in an analogue of the Schlemm canal were found in rabbits exposed to prolonged ATCL [26].

Given the aforementioned findings, we believe it is important to assess (1) the morphological and functional optic nerve changes in rabbits exposed to prolonged ATCL leading to hypopinealism and (2) the impact of a course of injections with melatonin on these changes.

The purpose of the study was to assess the impact of a course of injections with melatonin on the morphological and functional optic nerve changes in rabbits maintained under conditions of prolonged ATCL leading to hypopinealism.

Material and Methods

Adult rabbits were used in this experimental study which was conducted at the vivarium of the Danilevsky Institute for Endocrine Pathology Problems. All animal experiments were conducted in compliance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes from the European Treaty Series (Strasbourg, 1986), General Ethical Principles for Experiments in Animals, adopted by the First National Bioethics Congress (2001), and the Law of Ukraine on Protection of Animals from Cruel Treatment No. №3447-IV dated 21.02.2006.

Animals were divided into three groups. Group 1 (an ATCL group) was composed of 32 animals (64 eyes) exposed to ATCL (natural sunlight at daytime and 30-40-lux illumination from 100-W incandescent lamps at night time) to develop functional hypopinealism [23, 27]. Group 2 (an ATCL+M group) was composed of 29 animals (58 eyes) exposed to ATCL but treated with intramuscular (IM) melatonin (Sigma Chemical Co, St. Louis, Missouri; a 2.5 μg/kg/dose daily injected an hour before darkness) for 14 days immediately before being euthanized. Group 3 (a control group or CG) was composed of 23 intact animals (46 eyes) maintained under natural day/night cycle conditions.

Groups were subdivided into subgroups based on experimental constructs (Table 1). For example, the ATCL group included an ATCL1-2 subgroup, with the subgroup animals euthanized 1-2 months after the initiation of the experiment. Control subgroups were maintained under vivarium conditions for the same periods as the subgroups of groups 1 and 2.

Blood melatonin levels were assessed by commercially available enzyme-linked immunosorbent assay (ELISA) kits (IBL International, Hamburg, Germany). Photometric measurements were performed on an ELISA plate reader (Stat Fax 303 Plus, Awareness Technology Inc, Palm City, FL). All animals were euthanized by an overdose of sodium thiopental as per the guidelines from the Ministry of Health of Ukraine [28].

After the animal was euthanized, the eyes were enucleated, fixed in neutral formalin and routinely processed into paraffin-embedded blocks. Blocks were

Table 1. Division of experimental animals into groups and subgroups

ATCL		ATCL+M		CG	
Subgroups based on experiment time points	**Number of rabbits (eyes)**	**Subgroups based on experiment time points**	**Number of rabbits (eyes)**	**Subgroups based on experiment time points**	**Number of rabbits (eyes)**
$ATCL_{1-2}$	8 (16)	$ATCL+M_{1-2}$	5 (10)	CG_{1-2}	5 (10)
$ATCL_{3-5}$	9 (18)	$ATCL+M_{3-5}$	9 (18)	CG_{3-5}	5 (10)
$ATCL_{8-12}$	5 (10)	$ATCL+M_{8-12}$	5 (10)	CG_{8-12}	5 (10)
$ATCL_{18-19}$	5 (10)	$ATCL+M_{18-19}$	5 (10)	CG_{18-19}	4 (8)
$ATCL_{26-28}$	5 (10)	$ATCL+M_{26-28}$	5 (10)	CG_{26-28}	4 (8)
Total	23 (46)		29 (58)		23 (46)

Note: ATCL, around-the-clock-light group with no treatment; ATCL+M, around-the-clock-light group treated with melatonin; CG, control group

cut into 4-5-μm sections which were mounted on slides and stained with hematoxylin and eosin, Mallory's triple stain or van Gieson's picrofuchsin to reveal components of the connective tissue. Thereafter, low-magnification microscopy was used for general assessment of the optic nerve tissue, and morphological study was conducted [29, 30]. Histological and morphometric evaluations were carried out under a light microscope Olympus BX-41 (Olympus Europe GmbH, Munich, Germany) using appropriate software (Olympus DP-Soft 3.1, Olympus Europe GmbH) and Microsoft Excel [31]. Morphometric analysis was conducted using the field method [32].

Analysis of variance and alternative analysis were employed for statistical analysis. The mean, standard error of the mean, degree of dispersion, standard deviation and P values (significance of difference between time points) were calculated. A t-test was used to compare the means of two small samples [33]. P values < 0.05 were considered significant.

Table 2. Blood melatonin levels in animals at different experiment time points (M ± m)

Groups of animals	Blood melatonin levels in animals (pmol/l)	
	Day time	Night time
CG	54.41±6.15	369.45±14.35
$ATCL_{1-2}$	34.52±5.28*	66.23±4.87*
$ATCL_{3-5}$	29.12±5.85*	62.26±5.27*
$ATCL_{8-12}$	28.61±4.22*	60.53±5.19*
$ATCL_{18-19}$	32.22±5.49*	58.31±6.21*
$ATCL_{26-28}$	30.31±4.87*	59.42±5.63*

Note: *, significant difference ($p < 0.05$) compared to controls; ATCL, around-the-clock-light group with no treatment; CG, control group; M ± m, mean ± standard error of mean

Results

At 1 month after initiation of the experiment, blood melatonin level at daytime in the ATCL and ATCL+M groups decreased to 29.12 ± 5.85 pmol/l, which was statistically significantly lower than blood melatonin level in the control group (54.41 ± 6.15 pmol/l; $p < 0.05$). In addition, night-time blood melatonin level decreased to 62.26 ± 5.27 pmol/l (i.e., almost six-fold lower than that in the control group, 369.45 ± 14.35 pmol/l; $p < 0.05$) as early as month 1, and remained low at subsequent time points (Table 2).

At 1 to 2 months, signs of abnormal blood supply in the intraorbital optic nerve were seen in the ATCL1-2 and ATCL+M1-2 subgroups. Optic nerve sheath vessels appeared dilated or hyperemic (Fig. 1). In addition, along the course of vessels, there was accumulation of leakage in perivascular spaces, which at some sites resulted in capillary wall breakage from adjacent tissues (Fig. 2). Our morphometric study found that the mean relative vascular area (RVA) in the intraorbital optic nerve specimens was (3.49 ± 0.18) % for the ATCL+M1-2 subgroup, which was significantly different from the ATCL1-2 subgroup ((3.94 ± 0.19) %, $p< 0.05$), but not from the CG1-2 subgroup ((3.32 ± 0.19) %, $p > 0.05$). The mean relative area of the perivascular connective tissue (RAPCT) was (2.31 ± 0.13) % for the ATCL+M1-2 subgroup (Table 3), which was significantly different from the ATCL1-2 subgroup and the CG1-2 subgroup ((2.30 ± 0.12) % and (2.20 ± 0.19) %, respectively; $p > 0.05$). The mean diameter of the nerve fiber bundle (DNFB) was (4.37 ± 0.14)×10-6 m for the ATCL+M1-2 subgroup (Table 3), which was significantly ($p< 0.05$) shorter than for the ATCL1-2 subgroup ((4.89 ± 0.11)×10-6 m) and not significantly different ($p > 0.05$) from the CG1-2 subgroup ((4.32 ± 0.11)×10-6 m).

At 3 to 5 months, signs of marked abnormalities in the circulation of blood to the optic nerve were observed in the ATCL+M3-5 and ATCL3-5 subgroups, with markedly dilated and hyperemic vessels in the microcirculatory bed (MCB), abrupt perivascular space edema (Fig. 3), and

Table 3. Morphometric parameters of the intraorbital optic nerve in control and experimental groups at different time points of around-the-clock illumination and after a course of injections with melatonin

Subgroups	Mean DNFB (×10-6 m) M±m	Mean RVA (%) M±m	Mean RAPCT (%) M±m
CG_{1-2}	4.32±0.11	3.32±0.12	2.30±0.12
$ATCL_{1-2}$	4.89±0.11 [a]	3.94±0.19 [a]	2.20±0.19
$ATCL+M_{1-2}$	4.37±0.14 [d]	3.49±0.18 [d]	2.31±0.13
CG_{3-5}	4.38±0.13	3.51±0.14	2.35±0.13
$ATCL_{3-5}$	4.77±0.12	4.87±0.12 [a. b]	2.91±0.14 [a, b, c]
$ATCL+M_{3-5}$	4.31±0.13 [d]	4.12±0.17 [a, b, d]	2.40±0.12 [d]
CG_{8-12}	4.24±0.14	3.49±0.18	3.50±0.12
$ATCL_{8-12}$	3.17±0.11 [a, b, c]	3.65±0.17 [b]	4.30±0.13 [a, b, c]
$ATCL+M_{8-12}$	3.13±0.14 [a, b, c]	3.58±0.16 [b]	4.31±0.14 [a, b, c]
CG_{18-19}	3.89±0.11 [b]	3.23±0.17	3.50±0.15
$ATCL_{18-19}$	2.73±0.11 [a, b, c]	2.56±0.14 [a, b, c]	4.60±0.17 [a,b]
$ATCL+M_{18-19}$	2.83±0.12 [a, b, c]	2.67±0.12 [a, b, c]	4.51±0.15 [a]
CG_{26-28}	3.85±0.14	3.20±0.13	3.40±0.14
$ATCL_{26-28}$	2.51±0.09 [a, c]	2.01±0.15 [a, b, c]	4.80±0.15 [a, c]
$ATCL_{+M26-28}$	2.73±0.10 [a, c]	1.93±0.15 [a, b, c]	4.61±0.17 [a, c]

Note: M ± m, mean ± standard error of mean; a, significant difference ($p < 0.05$) compared to controls at the same time point; b, significant difference ($p < 0.05$) compared to the previous time point; c, significant difference ($p < 0.05$) compared to the ATCL 1-2 subgroup; d, significant difference ($p < 0.05$) compared to the ATCL subgroup at the same time point; DNFB, diameter of the nerve fiber bundle; RAPCT, relative area of the perivascular connective tissue; RVA, relative vascular area

accumulation of vasogenic leakage in the submeningeal spaces with peripheral compression of the neural tissue and subsequent myelin sheath disintegration in this region (Fig. 4). Hyperemia in vessels caused a significantly increased RVA in the intraorbital optic nerve in the ATCL3-5 subgroup compared to the CG3-5 subgroup ((4.87±0.12) % versus (3.51±0.14) %, $p < 0.05$). The mean RVA in the intraorbital optic nerve in the ATCL+M3-5 subgroup ((4.12 ± 0.17) %) was lower than in the ATCL3-5 subgroup ($p < 0.05$). The RAPCT in the ATCL+M3-5 subgroup was significantly lower than in the CG3-5 subgroup ((2.4 ± 0.12) % versus (2.91 ± 0.14) %, $p < 0.05$), but not than in the ATCL3-5 subgroup ((2.35 ± 0.13) %, $p > 0.05$). The DNFB in the ATCL+M3-5 subgroup was significantly lower than in the ATCL3-5 subgroup ((4.31 ± 0.13)×10-6 m versus ((4.77 ± 0.12)×10-6 m, $p < 0.05$), but not than in the CG3-5 subgroup (4.38 ± 0.13)×10-6 m, $p > 0.05$) (Table 3).

At 8 to 12 months, signs of abnormalities in the circulation of blood to the optic nerve decreased in severity in the ATCL+M8-12 and ATCL8-12 subgroups, with a reduction in vessel dilation, which was confirmed by morphometric analysis. The RVA in the intraorbital optic nerve specimens was (3.49 ± 0.18) % for the ATCL+M8-12 subgroup, which was significantly lower than at the previous time point in the ATCL+M3-5 subgroup ((4.12 ± 0.17) %, $p < 0.05$), and was not significantly different from the CG8-12 and ATCL8-12 subgroups (3.49 ± 0.18)% and (3.65 ± 0.17)%, respectively, $p > 0.05$). Neural perivascular spaces increased in volume at the expense of connective tissue neoplasms. In the ATCL+M8-12 subgroup, signs of perivascular sclerosis were confirmed by the RAPCT, which was significantly higher than at the previous time point in the ATCL+M3-5 subgroup ((4.31 ± 0.14) % versus (2.40 ± 0.12) %, $p < 0.05$), and was not significantly different from the CG8-12 subgroup ((4.30 ± 0.13) %, $p > 0.05$) (Table 3). The DNFB in the ATCL+M8-12 subgroup was significantly lower than in the CG8-12 subgroup ((3.13 ± 0.14)×10-6 m versus (4.24 ± 0.14)×10-6 m, $p < 0.05$), but not than in the ATCL8-12 subgroup ((3.17 ± 0.11)×10-6 m, $p > 0.05$) (Table 3).

Reduced vascular bed in the intraorbital optic nerve was observed in animals in the ATCL+M18-19 subgroup, with the RVA in the intraorbital optic nerve specimens being significantly reduced compared to the animals in the CG18-19 subgroup ((2.67±0.12) % versus (3.23 ± 0.17) %, $p < 0.05$, Table 3). Neural perivascular spaces were found to be increased in volume at the expense of connective tissue neoplasms. The RAPCT was (4.51 ± 0.15) % for the ATCL+M18-19 subgroup, which was significantly larger than for the CG18-19 subgroup (3.50 ± 0.15)%, $p < 0.05$), and not significantly different from

the ATCL18-19 subgroup ((4.60 ± 0.17) %, $p > 0.05$). The DNFB was (2.83 ± 0.12)×10-6 m for the ATCL+M18-19 subgroup, which was significantly ($p < 0.05$) smaller than for the CG18-19 subgroup ((3.89 ± 0.11)×10-6 m) and not significantly different ($p > 0.05$) from the ATCL18-19 subgroup ((2.73 ± 0.11)×10-6 m) (Table 3).

At 26 to 28 months, reduced MCB vascular bed in the intraorbital optic nerve was observed in animals in the ATCL+M26-28 subgroup, with the RVA in the intraorbital optic nerve specimens being significantly reduced compared to the animals in the CG26-28 subgroup ((1.93 ± 0.15) % versus (3.20 ± 0.13) %, $p < 0.05$, Table 3). Perivascular spaces in the optic nerve appeared widened due to sclerosis and filled with the connective tissue showing van Gieson's picrofuchsin staining (Fig. 5). The RAPCT in the ATCL+M26-28 subgroup was significantly higher than in the CG26-28 subgroup ((4.61 ± 0.17) % versus (3.40 ± 0.14) %, $p < 0.05$), and close to that in the ATCL26-28 subgroup ((4.80 ± 0.15) %, $p > 0.05$, Table 3). The DNFB in the ATCL+M26-28 subgroup was significantly lower than in controls ((2.73 ± 0.10)×10-6 m versus ((3.85 ± 0.14)×10-6 m, $p < 0.05$), but not than in the ATCL26-28 subgroup (2.51 ± 0.09)×10-6 m, $p > 0.05$) (Table 3).

Discussion

In modern societies, the problem of prolonged exposure to artificial light during hours of darkness is important, because a reduction in daily hours of darkness causes reduced pineal gland activity and melatonin synthesis and, consequently, affects human circadian rhythms and the antioxidant system of the body (in which melatonin plays a key role), leading to metabolic syndrome, etc. [5–8].

The impact of impaired circadian rhythms and melatonin synthesis on the eye is of special interest. First, the retina converts light stimuli into neural impulses which are transmitted to the CNS (particularly, the pineal gland) by the optic nerve. Second, melatonin is produced by retinal photoreceptors [10, 11] and ciliary body epithelium [12], and melatonin receptors are found throughout the ocular tissue, including the cornea, lens, ciliary body, retina, choroid and sclera [21]. The above findings indicate that the function and structure of the eye are closely associated with melatonin synthesis.

An experimental model of hypopinealism has been developed at the Danilevsky Institute for Endocrine Pathology Problems. The model is based on the fact that around-the-clock light exposure impairs the function of the pineal gland as a major melatonin supplier. In that experimental study, the development of hypopinealism in rabbits was demonstrated by reduced melatonin production and morphological changes in the pineal gland with reduced pineal gland cell counts [23, 25, 27]. Early atherosclerotic changes in the vasculature throughout the body and signs of early pineal gland aging have been reported [27]. Our previous studies on hypopinealism demonstrated degenerative changes in the retina, choroid [4], ciliary body and iris in rabbits [26].

These findings and endocrinological studies allowed us to hypothesize that rabbits with hypopinealism might develop optic atrophy.

As early as 1-2 months after the initiation of the experiment, in experimental rabbits, night-time melatonin levels in blood were almost six times higher than in controls, and daily melatonin production decreased almost half. However, the subsequent decrease in daily melatonin production was insubstantial, and this fact requires further research. It can be hypothesized that, in the presence of a suppressed pineal gland activity, the enterochromaffin cells of the rabbit gastrointestinal tract or other melatonin production-related compensatory processes become somewhat activated [9].

Animals with functional hypopinealism in the presence of ATCL exhibited the changes in the intraorbital optic nerve which were generally similar to those seen in the animals that received a course of injections with melatonin at different time points before being euthanized.

At 3 to 5 months of ATCL, signs of marked abnormalities in the circulation of blood to the optic nerve were seen, and optic nerve sheath vessels appeared dilated or markedly hyperemic. Perivascular space edema was so marked that capillary wall breakage from adjacent tissues was observed at some sites. At 18 to 12 months of ATCL, signs of abnormalities in the circulation of blood to the optic nerve gradually decreased in severity. At 12 to 28 months of ATCL, circulatory abnormalities in the optic nerve appeared to be changed by sclerotic and atrophic processes, and massive sclerosis of the preivascular spaces, abrupt vascular wall thickening and nerve-bundle thinning were observed. In addition, optic nerve sheaths appeared thickened and exhibited staining with fuchsin, indicating sclerosis. Throughout the experiment, in melatonin-deficient animals, the changes in the intraorbital optic nerve were significantly greater than age-related optic-nerve changes in rabbits of control subgroups.

Optic nerve demyelination was observed from months 3-5 of the experiment, and was seen at subsequent time points in animals exposed to ATCL, both treated and non-treated with melatonin. No optic nerve demyelination was observed in control animals.

In animals with hypopinealism, a course of injections with melatonin had some impact on the morphology and function of the intraorbital optic nerve, mostly at the early time points of the experiment. At 1-5 months, in animals exposed to ATCL and treated with melatonin, the signs of abnormalities in the circulation of blood were less severe than in animals exposed to ATCL and non-treated with melatonin. This was indicated by a significant melatonin-induced reduction in the mean relative vascular area in the intraorbital optic nerve and mean diameter of the nerve fiber bundle, whereas the increased nerve fiber thickness at these time points was associated with edema. At 3-5 months, in animals exposed to ATCL and treated with melatonin, there was a reduction in the mean relative area of the perivascular connective tissue, whereas the increase

in this parameter at previous time points is believed to be also associated with a reactive edema.

At 12-28 months, in animals exposed to ATCL and treated with melatonin, there were no significant changes in vascular sclerosis-related parameters and thickness of the nerve fiber bundle compared to animals exposed to ATCL and non-treated with melatonin. At these time points, in animals exposed to ATCL, both treated and non-treated with melatonin, there was an increase in parameter difference from animals in control subgroups, with a significant increase in the mean relative vascular area in the intraorbital optic nerve (due to low blood flow in vessels and vessel obliteration) and decrease in the mean relative area of the perivascular connective tissue.

Therefore, the morphological findings (like demyelination of nerve fibers and thinning of nerve fiber bundles of the optic nerve), combined with low blood flow in and obliteration of optic nerve vessels, vascular wall thickening and connective tissue growth, indicated the development of sclerotic atrophy of the optic nerve, in the presence of marked melatonin deficiency, in rabbits exposed to ACLI. On the basis of our previously reported findings of degenerative changes in the retina of rabbits from this experiment [4], we may suppose ascending OA in these rabbits. Findings of circulatory and degenerative abnormalities in the ciliary body and iris, and previous findings of newly formed rough bundles of collagen fibers in an analogue of the Schlemm canal in eyes of the rabbits [26] involved in this study, allow us to suppose abnormal hydrodynamics of the eye and the development of glaucomatous optic nerve atrophy in these rabbits. This is in agreement with findings on the involvement of melatonin in the control of intraocular pressure and the development of glaucoma [18]. Further research is required to clarify the pathomorphological aspects of OA associated with hypopinealism.

Experimental treatment of rabbits exposed to ATCL with melatonin aimed to find a method of medical correction of (a) hypopinealism and (b) optic nerve changes. The 14-day course melatonin treatment of ATCL-exposed rabbits immediately before their euthanasia exerted anti-edematous effects at early time points (< 5 months), until obviously irreversible changes in the optic nerve had occurred. However, the course of injections with melatonin exerted no impact on the development of OA in animals with persistent, marked hypopinealism developed in the presence of prolonged (28-month) exposure to ATCL. It can be hypothesized that, melatonin treatment for a period as long as the duration of the rabbit experiment with exposure to ATCL would be more effective for the prevention of OA than the 14-day course melatonin treatment, but this requires further research.

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