

Experimental studies

Effect of various single doses of intravitreal topotecan on the ultrastructure of the rabbit retina

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Вплив інтравітреальних ін'єкцій різних доз топотекану на ультраструктуру сітківки кроликів

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Abstract

Purpose. To assess ultrastructural changes in the rabbit retina after various single doses of intravitreal topotecan.

Material and Methods. Eyes of 12 Chinchilla rabbits were divided into an experimental group (20 eyes) and a control group (4 eyes). The former group was divided into five subgroups of four eyes each to receive an intravitreal injection (IVI) of 0.1 ml topotecan at a dose of 20, 30, 40, 50 or 60 µg. The control group was given an IVI of 0.1 ml saline. Animals were followed up the next day after injection and at weeks 1, 2, 3 and 4 to evaluate their general status and examine their eyes by slit lamp microscopy and direct ophthalmoscopy. At one month after injection, both eyes were enucleated and rabbits were euthanized by air embolism. Thereafter, chorioreti-

nal complex samples were examined by electron microscopy. The evaluation of ultrastructural changes in the chorioretinal complex was qualitative and descriptive in nature.

Results. The rabbits' general well being and general condition of the anterior segment of the eye were unaffected in all groups. Ophthalmoscopically, no pathological changes were observed after 20- to 40-µg injection. However, one week after 50- to 60-µg injection, there was evidence of mild retinal whitening which did not increase in severity at subsequent follow-up evaluations. Ultrastructural changes were dose dependent. After 20 to 30-µg injection, we observed mild reversible hydropic changes in retinal pigment epithelial cells and inner retinal layers, but the retina retained its layered structure. A 40 to 50-µg injection was followed by more apparent hydropic dystrophy of chorioretinal complex structures. After 60-µg injection, we observed initial alterations in individual neural organelles.

Conclusion. 20-30-µg intravitreal topotecan injection caused only mild reversible changes in retinal ultrastructure. A dose of 40 to 50 µg (equivalent to 80 to 100 µg in the human eye) may be considered a dosage that achieves an optimal efficacy/toxicity balance. A dose of 60 µg (equivalent to 120 µg in the human eye) caused initial alterations in the chorioretinal complex. Our findings (1) confirm that topotecan has a lower toxicity than melphalan and (2) may be used to justify the selection of a safe dose for intravitreal chemotherapy for retinoblastoma.

Keywords: intravitreal chemotherapy, topotecan; retinal ultrastructure, rabbits, experiment, retinoblastoma

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Резюме

Мета. Вивчити ультраструктурні зміни сітківки кроликів після інтравітреального введення топотекану в різних дозах.

Матеріал та методи. Дослідження проведено на 12 кроликах породи шиншилла. Десяти тваринам виконували одноразову інтравітреальну ін'єкцію 0,1 мл топотекану в дозах 20, 30, 40, 50 або 60 мкг (5 підгруп по 4 ока). Контрольній групі (2 тварини, 4 ока) вводили 0,1 мл фізіологічного розчину. Контрольні огляди тварин після ін'єкції проводилися наступного дня, через 1, 2, 3, 4 тижні після ін'єкції з оцінкою загального стану та стану очей: під щільною лампою та офтальмоскопічно. Через місяць після ін'єкції в умовах загальної анестезії проводилась енуклеація обох очей кроликів з наступним виведенням з експерименту шляхом повітряної емболії, після чого було проведено електронно-мікроскопічне дослідження хоріоретинального комплексу. Оцінка ультраструктурних змін хоріоретинального комплексу мала якісний описовий характер.

Результати. Загальний стан тварин та стан переднього відділу ока залишалися без змін у всіх групах. Офтальмоскопічно після введення 20–40 мкг топотекану патологічних змін не виявлено, після доз 50 і 60 мкг через 1 тиждень відзначалося незначне погіршення сітківки

без подальшого прогресування. Ультраструктурний аналіз показав дозозалежний характер змін. При введенні 20–30 мкг спостерігалися мінімальні оборотні гідропічні зміни клітин пігментного епітелію та внутрішніх шарів сітківки зі збереженням її архітектоники. Дози 40–50 мкг супроводжувалися більш вираженою гідропічною дистрофією структур хоріоретинального комплексу. При введенні 60 мкг виявлено початкові альтераційні зміни окремих органел нервових клітин.

Висновки. Інтравітреальне введення топотекану в дозах 20–30 мкг (еквівалент 40–60 мкг для людини) викликає лише мінімальні оборотні ультраструктурні зміни сітківки. Дози 40–50 мкг (еквівалент 80–100 мкг у людини) забезпечують оптимальне співвідношення потенційної ефективності та токсичності. Доза 60 мкг (еквівалент 120 мкг у людини) супроводжується початковими альтераційними змінами хоріоретинального комплексу. Отримані результати підтверджують нижчу токсичність топотекану порівняно з мелфаланом і можуть бути використані для обґрунтування вибору безпечних доз інтравітреальної хіміотерапії ретинобластоми.

Ключові слова: інтравітреальна хіміотерапія, топотекан, ультраструктура сітківки, кролики, експеримент, ретинобластома.

Introduction

Eye-sparing treatments are based on local and systemic chemotherapy and are the main approach to the treatment of retinoblastoma, even in advanced cases [1-4]. High efficacy with minimal toxicity is a determinant for the selection of a cytostatic agent for intravitreal chemotherapy (IVC). Until recently, melphalan was a standard of cytostatic agent for IVC for retinoblastoma. It displays a high antitumor activity, but its use is associated with the risk of toxic retinopathy, vasculopathy and irreversible visual function loss [5-8]. Therefore, the search for new effective drugs continues, and ophthalmologists have turned their attention to the cytostatic agent topotecan as an alternative for melphalan in the treatment of retinoblastoma. Topotecan has been developed as a semi-synthetic derivative of camptothecin, to specifically inhibit topoisomerase I. By stabilizing the DNA-topoisomerase I complex, topotecan prevents the repair of single-strand DNA breaks and during DNA replication causes cytotoxic damage to tumor cells. Topotecan is an anti-tumor agent used in ocular oncology for treating refractory or recurrent vitreous seeds in retinoblastoma. The major advantages of topotecan are a high concentration in the vitreous, almost complete absence of systemic effects, and potentially low toxicity [9-14]. Rabbit studies have been conducted to assess changes in electroretinography (ERG) and retinal histopathological response to intravitreal topotecan [15-16]. To the best of our knowledge, there have been no previous experimental studies evaluating the ultrastructure of a healthy retina after intravitreal injection of various doses of topotecan.

The purpose of this study was to evaluate ultrastructural changes in the rabbit retina after various single doses of intravitreal topotecan.

Material and Methods

Eyes of 12 Chinchilla rabbits (weight, 2.5–3 kg) were divided into an experimental group and a control group. Animals in the experimental group (10 rabbits) received an intravitreal injection (IVI) of 0.1 ml topotecan at various doses in both eyes, and were divided into five subgroups of two rabbits (4 eyes) each based on the dose injected (Table 1). The control group was given an IVI of 0.1 ml saline in both eyes.

Animals were maintained on a 12h light/dark cycle and fed and watered ad libitum. All animal experiments complied with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, France, 1986).

This experimental study was approved by the Bioethics Committee of SI “The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine” (committee minutes dated April 17, 2025).

All IVI were conducted in sterile conditions under general anesthesia. Animals were followed up the next day after injection and at weeks 1, 2, 3 and 4 to assess their general status and examine their eyes by slit lamp microscopy and direct ophthalmoscopy. At one month after injection, both eyes were enucleated and rabbits were

Table 1. Distribution of intravitreal topotecan doses among rabbit eyes

Subgroup	I	II	III	IV	V
Topotecan dose (µg)	20	30	40	50	60
Number of rabbit eyes	4	4	4	4	4

euthanized by air embolism. Posterior segment tissue samples were excised and processed routinely for electron microscopy analysis. In short, these samples were fixed using 2.5% glutaraldehyde in 7.4 pH phosphate-buffered saline, and post-fixed with 1% osmic acid in 7.4 pH phosphate-buffered saline. Sections were dehydrated with ascending grades of alcohol and embedded in Epon-araldite mixture. The material for histology was examined in a PEM-100-01 electron microscope (Selmi, Sumy, Ukraine). The nature and severity of morphological changes, if any, in the cellular and extracellular structures of the choriocapillaris and retina, were assessed. Because the quantitative assessment of morphological changes was not conducted, no statistical analysis of the ultrastructural characteristics was performed. Given the difference in the vitreous volume between the rabbit and human eyes (about 1.7–2.0 ml and 3.4–4.0 ml, respectively), a coefficient of 2:1 was used for approximate comparison of intravitreal doses between the human and rabbit eyes. Therefore, topotecan doses of 20, 30, 40, 50 and 60 µg in rabbits corresponded to about 40, 60, 80, 100 and 120 µg, respectively, in humans [9].

Results

No change in behavior or weight or hair loss was observed in the control and experimental groups at any follow-up point. All rabbits had no anterior segment changes and clear optic media on slit-lamp examination. Ophthalmoscopically, no retinal changes were observed in the control group and subgroups I, II and III after intravitreal injection of topotecan at a dose of 20, 30 and 40 µg, respectively. At one week after IVI of topotecan at a dose of 50 and 60 µg, rabbits in subgroups IV and V, respectively, had ophthalmoscopic evidence of mild retinal whitening with mild pigment redistribution which did not increase in severity at subsequent follow-up evaluations.

At one month after IVI of physiological saline, rabbits in the control group had microscopic evidence of a normal ultrastructure of the choriocapillaris and retina (Fig. 1).

At one month after IVI of topotecan at a dose of 20 µg, in subgroup I, there was mild retinal pigment epithelium (RPE) cell edema which extended extracellularly to the outer limiting membrane. The smooth endoplasmic reticulum (SER) in the RPE responded to topotecan by some dilation of SER vesicles, which was possibly associated with manifestations of the detoxification function of the SER. The cytoplasm of some RPE cells and cells in other retinal layers (particularly, Müller cells and especially

their processes at the inner limiting membrane (ILM)) had increased electron density, with small regions with dilated intercellular spaces between these cell processes. The processes of neurons in the inner plexiform layer showed signs of mild edema, but retinal ganglion cells appeared normal. The ground substance of the connective tissue in the choriocapillaris and Bruch's membrane appeared somewhat transparent (i.e., swollen ground substance), with no changes in the endothelial cells in the choriocapillaris.

At one month after IVI of topotecan at a dose of 30 µg, in subgroup II, all retinal layers appeared well preserved. However, SER vesicles appeared sparsely arranged, with areas of vesicle destruction in RPE cells; other retinal abnormalities included focal disorganization of photoreceptor outer segments, mild hydropic changes in the structure of other retinal layers, and small regions with dilated intercellular spaces between Müller cell processes at the ILM. Not only the connective tissue, but also the endothelial cells in the choriocapillaris were edematous (Fig. 2).

At one month after IVI of topotecan at a dose of 40 µg, in subgroup III, the layered structure of the retina was intact. Some RPE cells had no apical microvilli and there was evidence of (1) edema in the interreceptor matrix and photoreceptor layer, and (2) more severe hydropic changes in the neural cells and their processes in the internal retina than those observed after an injection of a smaller dose of topotecan. Of note were some Müller cell processes at the ILM showing focal regions of osmiophilic cytoplasm, dilated SER vesicles and dilated intercellular spaces. In the choriocapillaris layer, there was evidence of hydropic dystrophy of endothelial cells and edematous ground substance of the connective tissue with sparsely arranged collagen fibrils and focal destruction of the latter (Fig. 3).

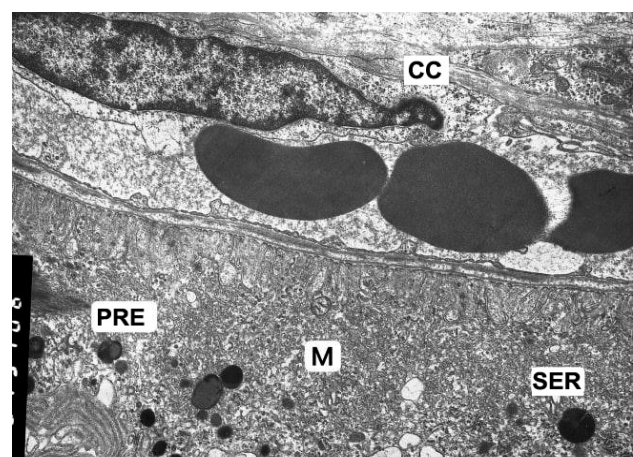


Figure 1. Electron micrograph (original magnification, ×8000). Ultrastructure of rabbit retina one month after a single intravitreal injection of 1 ml saline. The structures of choriocapillaris (CC), smooth endoplasmic reticulum (SER) vesicles and retinal pigment epithelium (RPE) cell mitochondria (M) appear normal.

At one month after 50- μ g IVI of topotecan in subgroup IV, and after 60- μ g IVI of topotecan in subgroup V, there were changes in the retina and choriocapillaris were similar to those observed after 40- μ g IVI of topotecan, but with sites of more severe hydropic changes in the retinal layers (e.g., the ganglion cell layer). Additionally, some sites near the ILM showed not only extracellular edema and more dilated intercellular spaces than after 40- μ g IVI of topotecan, but also damage to individual neural axons (Figs. 4–6).

Therefore, after 20- μ g or 30- μ g IVI of topotecan, the architecture of the retinal layers was generally not affected, but there was mild extracellular edema in the outer retinal layers and mild intracellular hydropic changes, with practically no changes in the structure of other retinal layers. The structures of the choriocapillaris layer reacted to topotecan administration, which is likely to be associated with the penetration of the drug from the vitreous into the ciliary body and choroidal circulation. Changes in the retinal structures gradually increased in severity with an increase

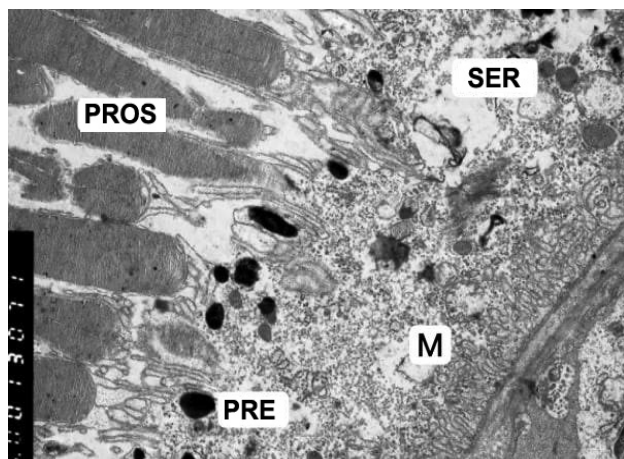


Figure 2. Electron micrograph (original magnification, $\times 6000$). Ultrastructure of rabbit's chorioretinal complex one month after a single intravitreal injection of 1 ml topotecan at a dose of 30 μ g. Note focal destruction of smooth endoplasmic reticulum (SER) vesicles, disorder of retinal pigment epithelium (RPE) cell mitochondria (M) and phagocytosis of photoreceptor outer segments (PROS).

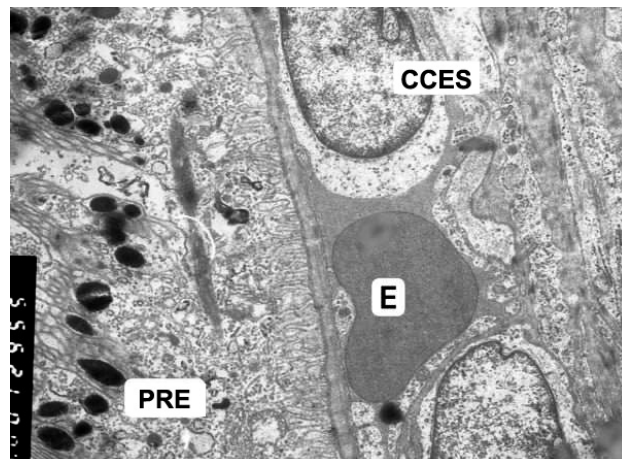


Figure 3. Electron micrograph (original magnification, $\times 5000$). Ultrastructure of rabbit's chorioretinal complex one month after a single intravitreal injection of 1 ml topotecan at a dose of 40 μ g. Note hydropic dystrophy of epithelial cells of choroidal capillaries (CCEC), an erythrocyte (E) in the capillary lumen and alterations in membranous organelles of retinal pigment epithelium (RPE) cells..

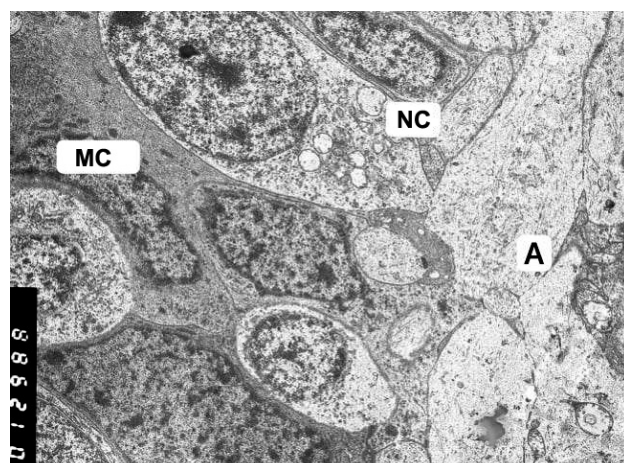


Figure 4. Electron micrograph (original magnification, $\times 5000$). Ultrastructure of rabbit's chorioretinal complex one month after a single intravitreal injection of 1 ml topotecan at a dose of 50 μ g. Note vacuolated mitochondria of a nerve cell (NC) located close to normal Müller cells and transparent axoplasm of axons (A).

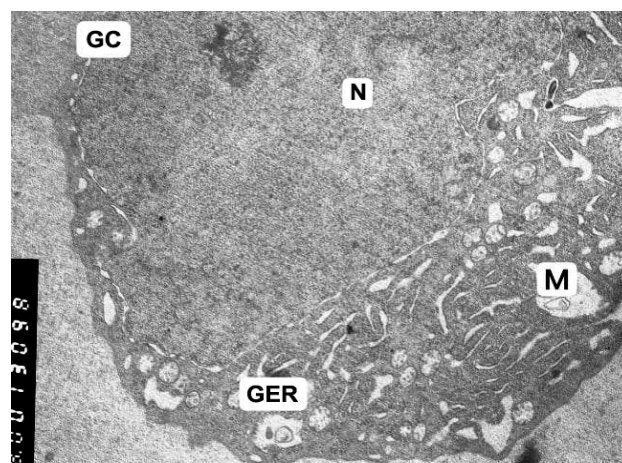


Figure 5. Electron micrograph (original magnification, $\times 8000$). Ultrastructure of rabbit's chorioretinal complex one month after a single intravitreal injection of 1 ml topotecan at a dose of 60 μ g. Note dilated cisternae of the granulated endoplasmic reticulum (GER) and vacuolated mitochondria (M) in ganglion cell (GC) cytoplasm and caryoplasm (N) of somewhat increased electron density.

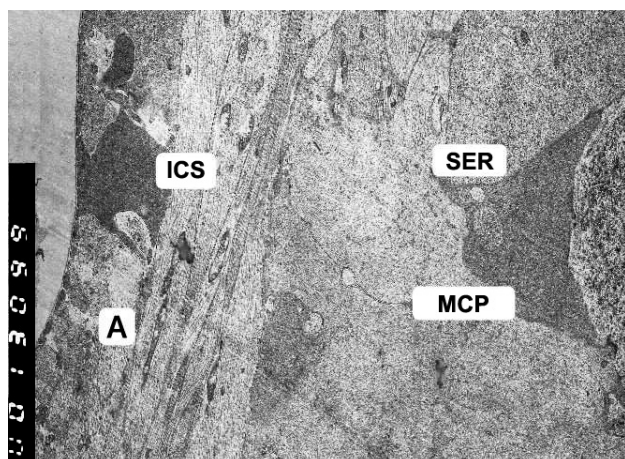


Figure 6. Electron micrograph (original magnification, $\times 4000$). Ultrastructure of rabbit's chorioretinal complex one month after a single intravitreal injection of 1 ml topotecan at a dose of 60 μg . Note dilated intercellular spaces (ICS), cytoplasm of increased electron density, dilated cysternae of the smooth endoplasmic reticulum (SER) and destruction of organelles of individual neural axons (A).

in the topotecan dose from 40 μg to 60 μg , and involved also ganglion cells after administration of the highest dose (60 μg).

Hence, with an increase in the topotecan dose from 40 μg to 60 μg , there was microscopic evidence of reactive changes in the SER at retinal sites near the ILM, with Müller cell processes having increased electron density, which was likely associated with the penetration of the drug into the cytoplasm of these cells. Moreover, we observed dilated intercellular spaces (i.e., the presence of intercellular edema) between these processes, and, after 60- μg IVI of topotecan, despite the aforementioned changes, there was evidence of destruction of individual neural axons.

The 20- μg to 60- μg topotecan doses used in the rabbit experiment are equivalent to 40- μg to 120- μg topotecan doses in the human vitreous and an increase in the topotecan dose caused an increase in the severity of hydropic changes with signs of initial alterations in the organelles of the chorioretinal complex. These changes are, however, reversible, and, in the absence of a damaging factor, the structure of these organelles may return to normal over time.

Discussion

Eye-preserving chemotherapeutic treatments for retinoblastoma have been increasingly used, partially due to the introduction of new targeted drug delivery techniques and changes in the selection of cytostatic drugs. Effective use of intravitreal topotecan in retinoblastoma has been increasingly reported [10-14]. Intravitreal topotecan is considered as an alternative for melphalan in the treatment of vitreous seeds of retinoblastoma due to the ability of the drug to achieve high local concentrations in the vitre-

ous with minimal systemic exposure and potentially lower retinal toxicity. However, the effects of intravitreally injected topotecan on the ocular tissues and most notably on normal retinal cells, have not yet been sufficiently studied. Experimental preclinical rabbit studies have provided topotecan that is currently used in clinical practice.

Buitrago and colleagues (2013) [15] studied the pharmacokinetics of topotecan after a single 0.5 μg intravitreal dose and evaluate the clinical and retinal toxicity after four weekly injections of two different doses of intravitreal topotecan (5 and 0.5 $\mu\text{g}/\text{dose}$) to the right eye of each rabbit. Animals were weekly evaluated for clinical and hematologic values (including topotecan concentrations in plasma and vitreous samples) and ocular evaluations were performed to establish potential topotecan toxicity. ERGs were recorded before and after topotecan doses. Despite multiple injections of 0.5 μg of topotecan are also safe to the rabbit eye, lactone topotecan vitreous concentrations were potentially active only after 5 h of the administration. Eyes injected with four weekly doses of topotecan (0.5 or 5 $\mu\text{g}/\text{dose}$) showed no significant differences in their ERG wave amplitudes and implicit times in comparison with control ($p > 0.05$). Animals showed no weight, hair loss or significant changes in hematologic values during the study period. No topotecan could be detected in plasma, which indicated almost no systemic exposure. The authors concluded that four weekly 5- μg IVI of topotecan are safe for the rabbit eye. They postulated promising translation to clinics for retinoblastoma treatment [15].

Bogan and colleagues (2022) [9] assessed the efficacy and toxicity of topotecan monotherapy (15 μg or 30 μg) in the treatment of WERI-Rb1 cell xenografts (RB vitreous seeds) in vivo in rabbits. Intravitreal topotecan 30 μg (equals 60 μg in humans) achieved the 90% inhibitory concentration (IC90) across the rabbit vitreous. Three weekly topotecan injections (either 15 μg or 30 μg) caused no retinal toxicity in rabbits, whereas melphalan 12.5 μg (equals 25 μg in humans) reduced ERG amplitudes 42%–79%. Intravitreal topotecan 15 μg was equally effective to melphalan to treat WERI-Rb1 cell xenografts in rabbits (96% reduction for topotecan vs saline ($p=0.004$), 88% reduction for melphalan vs saline ($p=0.004$), topotecan vs melphalan, $p=0.15$).

In a study by del Sole and colleagues (2022) [16], non-tumor-bearing rabbits received four weekly injections of three doses of topotecan (10 μg , 25 μg , and 50 μg) or vehicle alone. Eyes were examined by ophthalmoscopy and ERG, plasma and vitreous topotecan concentrations were determined, general clinical status was evaluated weekly, and retinal histology was studied to compare effects of the above doses. Eyes injected with all topotecan doses showed no signs of anterior segment inflammation, clinical or histologic evidence of damage to the retina, and ERG parameters remained unaltered throughout the study. Weight, hair loss, or changes in hematologic values were absent during the study period across all animal groups. It

was concluded that (1) four weekly IVI of topotecan up to 50 µg in the animal model or a 100 µg human equivalent dose were not toxic for the rabbit eye and (2) high doses of topotecan may show promising translation to the clinic for the management of difficult-to-treat retinoblastoma vitreous seeds.

Our studies on the same intravitreal topotecan doses as in aforementioned studies [9, 16] also demonstrated that they had no effect on the rabbits' general well being and general condition of the ocular structures. However, unlike previous studies, we used the analysis based on findings of electron microscopy, which found a difference in microstructural changes in the chorioretinal complex depending on the dose, even after a single dose. Thus, we found that an intravitreal dose of 20 µg or 30 µg in the rabbit (equivalent to 40 or 60 µg, respectively, in the human vitreous) led to functional changes in the retina, whereas that of at least 40 µg led to hydropic retinal dystrophy.

Del Sole and colleagues (2026) [17] used 24 rabbits divided into five groups of nontumor-bearing rabbits to evaluate systemic and ocular toxicity of 3 monthly doses of topotecan (50 µg, 100 µg, and 200 µg), injected in 50 µL or in 100 µL of diluent. Safety assessment included clinical monitoring of vital signs, hematologic values, ophthalmoscopy, fundus photography, ERG, and retinal histological examination. Three monthly IVI of topotecan 50 or 100 µg (100 µg and 200 µg human equivalent doses) caused no systemic or ocular toxicity in a nontumor-bearing rabbit model. Eyes injected with 200 µg showed localized retinal alterations near the injection site, and a significant decrease in the a- and b-wave amplitudes on ERG compared with the other groups ($P < 0.05$). Therefore, the authors determined the safety margin for intravitreal topotecan and concluded that three monthly IVI of topotecan 50 or 100 µg were safe, whereas repeated 200-µg doses may lead to local retinal toxicity.

The largest intravitreal topotecan dose that we assessed was 60 µg (a 100 µg human equivalent dose). This dose (but not a dose of 50 µg) led to cytoplasmic edema with alterations in neural organelles. Additionally, this dose is equivalent to 100 µg in humans; in our opinion, it would be optimal for the treatment of retinoblastoma because it is the dosage that achieves a balance between efficacy and toxicity.

We compared the effects of the same doses of intravitreal melphalan and intravitreal topotecan in the rabbit retina and found that the latter drug was significantly less toxic for the rabbit retina than the former drug. Our study [18] on the ultrastructural changes in the rabbit retina after various one-time doses (5, 10, 20, 30 and 40 µg) of intravitreal melphalan demonstrated a direct dose-dependent relationship with clinical and ultrastructural changes in the retina. After a one-time injection of 10 µg melphalan, there were mild ophthalmoscopic changes (a few small isolated sites of slight depigmentation) and ultrastructural changes (destructive changes at some retinal RPE locations and in

the layers and membrane discs of the photoreceptor outer segments and Müller cells). Additionally, after a single injection of 20 µg melphalan, we observed retinal changes in the form of sites of pigment redistribution and depigmentation, and ultrastructural changes presenting as disorganized retinal layers with abnormal ultrastructure of retinal photoreceptors and neurons, and Müller cell gliosis with destruction of intracellular structures or necrosis. Moreover, after a single injection of 30 µg or 40 mg melphalan, we found damage to all retinal layers and the choriocapillaris layer with glial replacement. The aforementioned findings are significantly different from those obtained in the present study on topotecan, which showed a significantly better safety profile.

Bar-Sela and colleagues [19] examined clinical and histological changes in the rabbit retina after a single dose (5, 15, 30 or 60 µg) of intravitreal melphalan. The eyes injected with 5 µg melphalan appeared normal, while the eyes treated with higher melphalan doses showed evidence of sclerotic retinal vessels and retinal whitening. Dose-dependent retinal structural damage was most apparent in the central retina close to the optic disc where the melphalan had been injected. The retinas of the experimental eyes, treated with melphalan dose of 5 µg, 15 µg, and 30 µm, exhibited a dose-dependent thinning of the inner nuclear layer and the outer nuclear layer. In contrast, the retina from the experimental eye injected with 60 µg, completely lost its layered organization indicating severe structural damage.

Conclusion

Experimental studies of the ultrastructure of the rabbit retina after various single doses (20, 30, 40, 50 and 60 µg) of intravitreal topotecan found the following:

First, there was no toxic effect of any of the aforementioned topotecan doses on the rabbit's general well-being and clinical condition of the rabbit's eye, aside from minimal ophthalmoscopic signs after injection of a large (50 or 60 µg) dose of the drug, with no increase in severity of the signs over the follow-up period.

Second, an intravitreal dose of 20 µg or 30 µg in the rabbit (equivalent to 40 or 60 µg, respectively, in the human vitreous) led to mild hydropic changes in the retina, whereas that of 40 µg or 50 µg led to hydropic retinal dystrophy, and that of 60 µg led to signs of initial alterations in the organelles of the chorioretinal complex. These changes are, however, potentially reversible, and, in the absence of a damaging factor, the structure of these organelles may return to normal over time.

Third, larger intravitreal doses of topotecan compared to melphalan are required to cause similar dystrophic changes in the retina, as evidenced by the literature and our previous study [9].

Finally, a topotecan dose of 40 to 60 µg (equivalent to 20 to 30 µg in the rabbit eye) may be considered conditionally safe for the human eye, whereas a dose of 80

to 100 µg (equivalent to 40 to 50 µg in the rabbit eye) may be considered a dosage that achieves an optimal efficacy/toxicity balance. Additionally, a topotecan dose of 120 µg (equivalent to 60 µg in the rabbit eye) can cause alterations in the chorioretinal complex; consequently, this dosage should be considered dangerous for humans even when administered experimentally.

Author contributions

The authors NB, TS, NM, AT and leV confirm their full responsibility for the following: conception and design of the study, collection of data, analysis and interpretation of the results, and preparation of the manuscript. All authors reviewed the results and approved the final version of the manuscript.

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Disclaimer

The views presented in this article are those of the authors and do not necessarily represent those of their institution.

Ethical Statement

This study was conducted on animals. All animal experiments complied with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, France, 1986). The experimental study was approved by the Bioethics Committee of SI "The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine" (committee minutes dated April 17, 2025)

Conflict of interest

The authors state that they have no conflict of interest that could influence their view on the subject matter or materials described and discussed in this manuscript.

Data Availability Declaration

All the data obtained or examined during this study has been incorporated into this published article.

Abbreviations

ERG, electroretinography; ILM, inner limiting membrane; IVC, intravitreal chemotherapy; IVI, intravitreal injection; RPE, retinal pigment epithelium; SER, smooth

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