Review

OCULAR PHARMACOLOGY OF TOPOTECAN AND ITS ACTIVITY IN RETINOBLASTOMA

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Purpose: To review the ocular pharmacology and antitumor activity of topotecan for the treatment of retinoblastoma by an evaluation of different routes of administration.

Methods: Systematic review of studies available at PubMed using the keywords retinoblastoma, topotecan, and camptothecins, including preclinical data such as cell lines and animal models, as well as clinical studies in patients with retinoblastoma.

Results: Forty-two available studies were reviewed. Evidence of antitumor activity against retinoblastoma as a single agent is based on data on cell lines and a limited number of affected patients with intraocular and extraocular disease when given in a protracted schedule. Evidence of additive or synergistic activity in combination with other agents such as carboplatin, melphalan, and vincristine was reported in preclinical and clinical models. In animal models, pharmacokinetic evaluation of topotecan administered by the periocular route shows that most of the drug reaches the vitreous through the systemic circulation. Topotecan administered by intravitreal injection shows high and sustained vitreal concentrations with limited systemic exposure and lack of retinal toxicity at a dose of up to 5 μg. Topotecan administered intraophthalmic artery shows higher passage to the vitreous compared with periocular administration in a swine model.

Conclusion: Topotecan alone or in combination is active against retinoblastoma. It shows a favorable passage to the vitreous when given intravenously and intraarterially, and ocular toxicity is minimal by all routes of administration. However, its clinical role, optimal dose, and route of administration for the treatment of retinoblastoma are to be determined.

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Retinoblastoma is the most frequent intraocular tumor of childhood and it is highly curable when diagnosed timely as usually happens in affluent societies. However, out of an estimated 8,000 children diagnosed with retinoblastoma worldwide each year, ~5,000 are diagnosed in developing countries, and a high proportion of them would die of disseminated disease.[1] Chemotherapy was historically used for the treatment of extraocular retinoblastoma; however, since the mid 90s,[2] intravenously administered multiagent chemotherapy using carboplatin-based regimens has become the standard conservative therapy.[3] Though highly effective for the treatment of eyes with less advanced disease, massive vitreous or subretinal seeds are difficult to control, and external beam radiotherapy or enucleation of the affected eye may be necessary.[4] Therefore, new therapeutic alternatives for the treatment of these eyes have been explored by different groups. One option would be to intensify systemic chemotherapy to achieve higher intraocular concentrations.[5] A limitation of this approach is the relatively poor penetration of drugs from the blood to the target ocular structures because of the blood-retinal barrier.[6] Increasing the systemically administered chemotherapy dose will conceivably increase systemic toxicity that is not recommended in these children receiving chemotherapy treatment with the purpose of preserving their eyes who have little or no direct risk of death caused by their tumor. Hence, drugs directed to different targets or innovative routes of administration have been explored to improve the ocular drug delivery while minimizing the systemic drug exposure.

The identification of active chemotherapy agents for retinoblastoma typically included a very limited number of Phase I-II studies recruiting patients with extraocular disease.[7,8] Because single-agent chemotherapy used upfront is rarely curable in this malignancy, clinical studies involving single drugs are scarce and usually included heavily pretreated relapsed patients. In addition, multicentric studies, essential to determine the role of new chemotherapy agents, have been difficult to implement for conservative treatments in retinoblastoma.[9]

Drug screening by in vitro chemosensitivity assays or animal models was done[10,11] but relatively few agents identified this way have been used in clinical practice. This approach also faces some limitations such as a different high expression of multidrug resistance that affects chemosensitivity of commercial cell lines[12] or differences in tumor biology of animal models because tumors in transgenic animals do not entirely recapitulate the human disease.[13–15] Specifically for retinoblastoma, in addition to assessment of antitumor activity, ocular pharmacokinetics of candidate drugs is critical for the evaluation of their clinical use. As vitreous seeding is one of the most important obstacles to cure intraocular retinoblastoma,[16] the major challenge is to find active drugs with good penetration into the vitreous. Because procurement of the vitreous specimens from patients with retinoblastoma for pharmacokinetic studies is not possible, the ocular pharmacologic data are inferred from animal models. Nevertheless, interspecies differences in the anatomy and physiology of the eye may limit the translation of these findings to humans. Hence, all these factors should be considered in the selection of candidate drugs for retinoblastoma. Our group and others pursued the evaluation of topotecan as a candidate drug for the treatment of retinoblastoma. Among the advantages of topotecan, its relatively low extrahematopoietic toxicity profile and its good diffusion through biological barriers such as the blood–brain barrier are attractive for clinical use in retinoblastoma.[17] Unlike other drugs used for retinoblastoma such as etoposide,[16] topotecan has not been conclusively associated with secondary leukemias. In addition, topotecan has been used by alternative routes of administration, such as intrathecally, including in a limited number of children with retinoblastoma in early clinical trials,[18] which would be of interest for the treatment of disease disseminated to the central nervous system.[19]

The pharmacokinetic profile of topotecan given by different routes of administration and animal and human evidence of activity against retinoblastoma were assessed and are the subject of this review.

Search Strategy

Studies were identified by a literature search through PubMed using the MeSH terms retinoblastoma, topotecan, and camptothecins. All the articles retrieved (accessed by March 12, 2014) were analyzed critically. The search yielded a total of 84 articles. After excluding 36 publications that did not refer to retinoblastoma but to the retinoblastoma gene on other tumors, 48 articles referring to the subject of our review were identified. Of them, 6 publications were excluded because they were reviews not reporting the original data (n = 3), clinical case reports (n = 2), or an article related to the technique of intraarterial chemotherapy (n = 1), not dealing directly with topotecan retinoblastoma treatment. Hence 42 publications were selected for this review.

Antitumor Activity of Topotecan Against Retinoblastoma

The first evidence of clinical activity of topotecan for retinoblastoma was reported in single cases in early clinical studies including children with a variety of tumors.[20,21] Subsequently, a series of 9 patients (6 extraocular, 3 intraocular) treated compassionately with 2 cycles of single-agent topotecan at 2 mg/m^2 per day for 5 consecutive days was reported.[22] A partial response was achieved in 3 of 6 patients with extraocular disease and in 2 of 3 with intraocular disease. Laurie et al[23] subsequently reported on the antitumor activity and ocular pharmacokinetics of topotecan alone or in combination with other drugs in cell lines and animal models. They reported that the most active combination was topotecan and carboplatin based on in vitro studies. However, the concomitant administration of both agents through intravenous infusion is precluded because of the hematologic toxicity elicited

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by the drugs in combination.[24] This study for the first time reported the 50% inhibitory concentration of topotecan (IC50) and determined the ability of the drug to pass into the vitreous.[23] Based on these results, the same group further explored the combination of topotecan and carboplatin. Nemeth et al[25] compared the antitumor activity of combined systemic topotecan and subconjunctival carboplatin with that of subconjunctival topotecan and systemic carboplatin in an orthotopic xenograft and in a knockout mouse model of retinoblastoma (Chx10-Cre; $Rb^{lox/lox}$; $p107^{-/-}$; $p53^{lox/lox}$).[25] This study showed that the preferred combination for translation into clinical practice was subconjunctival carboplatin concomitant to systemic topotecan.[25] Pharmacokinetic results indicated that regarding systemic exposure, topotecan penetration into the eye was better than that of carboplatin after subconjunctival injection because the area under the curve $(AUC)_{vitreous}/AUC_{plasma}$ was 1.98 and 0.85 for topotecan and carboplatin, respectively. In addition, an interesting finding was that in an orthotopic animal model, these ratios increased 3-fold and 1.5-fold compared with the nontumor-bearing animals, suggesting disruption of the blood-retinal barrier by the tumor. Another important finding was that none of the animals treated with subconjunctival topotecan (10 μg per eye) and systemic carboplatin (10 mg/kg) survived this experiment, and all died of toxicity. The authors reported that both drugs, when given periocularly, had evidence of systemic distribution, probably accounting for the systemic toxicity of the combination.[25] The animals receiving the higher systemic topotecan dosage showed significant tumor response in terms of reduced tumor burden and substantial restoration or preservation of vision with survival till 1 year of follow-up.[25] Based on these findings, a clinical Phase II study to evaluate efficacy and toxicity in patients with advanced intraocular retinoblastoma after 2 courses of vincristine and a topotecan window therapy was performed.[26] Topotecan was given in a protracted schedule, as suggested for other tumors,[27,28] in a short 30-minute daily infusion for 5 consecutive days, beginning with a fixed dose of 3 mg/m^2, repeating the same schedule 1 week apart. Subsequent doses were adjusted by pharmacokinetic parameters with a target topotecan systemic exposure of 140 ± 20 ng·h/mL and further escalated or deescalated in a personalized fashion. Depending on the clinical response to window therapy, patients received subsequent courses of chemotherapy consisting of vincristine–topotecan or only carboplatin-based regimens.[26] The combination of topotecan and vincristine achieved a partial response in almost 89% of patients with Reese–Ellsworth Group IV or V eyes; however, hematologic toxicity was significant including Grade 4 neutropenia in all patients, and 74% of the patients required transfusions. Although pharmacokinetically guided dosing is an important tool for attaining the target systemic exposure, this strategy is difficult to generalize in most centers lacking topotecan analytical assay and pharmacokinetics training for dose adjustment.

The association of topotecan and carboplatin by intraarterial administration was also studied.[29,30] When trying to identify new combination agents feasible for association with topotecan, studies in cell lines have found a synergistic effect of the association of topotecan and melphalan as well, which has been used piloted clinically.[31,32] Nevertheless, there is no clinical evidence that these associations result in enhanced antitumor activity because no studies have compared them to single drugs. Topotecan was also explored in preclinical models in combination with a p53-targeted therapy consisting of an ocular formulation of nutlin-3a that was added to the combination of systemic topotecan–periocular carboplatin. This study found an additive efficacy in knockout models that was, however, less evident in xenografts with human retinoblastoma.[33] In addition, an antiangiogenic effect of topotecan in the retinal vessels of nontumor-bearing newborn rats[34] and a pro-apoptotic effect of campthothecins by activation of FOXO1[35] and other mechanisms[36] were found in cell lines, all of which may also have therapeutic implications for retinoblastoma. Multidrug resistance protein modulators may further enhance topotecan antitumor activity in retinoblastoma cell lines.[37] Topotecan has also been a component of high-dose chemotherapy, and multidrug regimens were used for the treatment of extraocular disease.[38]

In conclusion, the activity of topotecan against retinoblastoma is evident in in vitro and in vivo models, and in patient cohorts with intraocular disease and a limited cohort of extraocular disease, yet its exact role in the clinical management of this tumor remains to be elucidated.

Ocular Pharmacology of Topotecan

Topotecan has been administered by different routes (Figure 1) for the treatment of retinoblastoma. Ocular pharmacology studies were done in animal models; however, it is important to stress that none completely resembles the clinical situation. Nontumor-bearing animals provide limited results because the blood-retinal barrier is intact in these animals. In clinical settings and in transgenic animals, there is a disruption of the blood-retinal barrier because of the disease that may favor the ocular penetration of chemotherapy.[39]

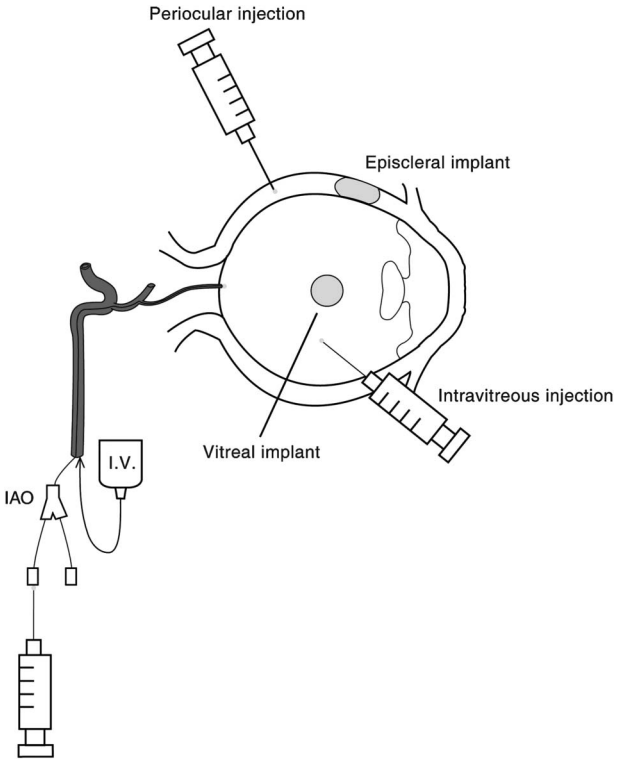

Fig. 1. Schematic representation of the different routes for topotecan administration for the treatment of retinoblastoma. IAO, intraarterial ophthalmic artery; IV, intravenous.

Differences in the size and volume of the eye, in the thickness of different ocular tissues such as the sclera, and in the fat content of the orbit, as well as the different structures of the retinal vessels may also introduce bias for these anatomo-physiologic differences between species. A comparison of the pharmacokinetic parameters of different routes of topotecan administration is shown in Table 1.

Pharmacokinetic parameters used for assessing the amount of drug that is available in the target site include the area under the concentration-versus-time curve which allows the investigator to estimate the potential therapeutic efficacy of the drug. The systemic AUC is, however, an indicator of adverse events such as myelosuppression. Another commonly used parameter is the C_{max}, the maximum concentration the agent reaches in the target tissue. A critical aspect for attaining clinical efficacy in retinoblastoma treatment is to ensure that the drug reaches the intended target site for an interval of time at concentrations above a certain threshold of cytotoxicity or drug activity. The IC50 calculated from in vitro studies of cytotoxicity may be an indicator of the minimum amount of the drug needed to reach the target tissue. Hence, understanding the pharmacokinetics of the drug according to the route of administration may allow for the determination of a rational dose and treatment scheme. These variables need to be related to the pharmacodynamic parameters that reflect the efficacy and safety of the treatment.

Periocular Administration

Periocular injection of chemotherapy is an option for delivering chemotherapy to the posterior segment of the eye, specifically for vitreous seeds.[40] An advantage of this route is that the drug theoretically follows a transscleral passage and, through the choroid and retina, may reach the target tissue circumventing the blood-retinal barrier. However, orbital clearance of the drugs after injection into the periocular space should be avoided. This strategy was initially studied for carboplatin delivery in children with retinoblastoma showing a favorable transscleral passage compared with intravenous administration in animals.[41] Pharmacokinetic studies in nontumor-bearing animals showed that topotecan was able to reach the vitreous after periocular administration, but its vitreal penetration was preferentially through the blood-retinal barrier rather than through the transscleral route.[42] Nevertheless, with the aim of evaluating topotecan as a candidate agent to be given periocularly in association with systemic carboplatin, a Phase I study of periocular topotecan was subsequently performed.[43] In that study, a total of 7 eyes of 5 patients with relapsed/resistant retinoblastoma facing imminent enucleation were evaluated using escalating doses from 0.5 mg to 2 mg for up to 2 courses. The dose-limiting toxicity was not achieved, and a maximum dose of 2 mg of periocular topotecan was well tolerated.[43] No significant toxicity was reported. A linear relationship between lactone topotecan AUC in plasma and dose, with a median (range) exposure of 36.6 ng·h/mL (12.7–54.2 ng·h/mL) was found. The systemic AUC was lower than 55 ng·h/mL in all cases, which is ~3 times lower than that reported to cause hematologic toxicity in pediatric patients.[28] Nevertheless, only one of seven of these heavily pretreated eyes was preserved after receiving this treatment and sequential topotecan-containing intravenous chemotherapy. To maximize the transscleral route while limiting the orbital clearance of the drug, delivery systems were explored.[44] A unidirectional and coated sustained-release preparation was designed and developed by Carcaboso et al[45] who created a biocompatible polymer-based implant loaded with higher (2.3 mg) or lower (0.3 mg) amounts of topotecan. Because previous experience suggested a role of the orbital vasculature in the clearance of topotecan, adrenaline was

Table 1. Pharmacokinetic Parameters of Topotecan Administered by Different Routes in Different Animal Models

Route	Dose, mg	Plasma			
		C_{max}, L, ng/mL	AUC, L, ng·h/mL	C_{max}, T, ng/mL	AUC, T, ng·h/mL
Animal model: rabbit					
Periocular	1	80 (62–98)	108.8 (4.8)	160 (110–210)	356.9 (8.8)
Intravenous	1	165 (140–205)	143.3 (4.1)	275 (240–320)	362.8 (8.2)
Intravitreal	0.005	—*	—*	21.1 (19.9–21.2)	469
Animal model: pig					
SSOAI	1	—*	—*	8.1 (7.4–9.5)	10.6 (6.8–13.4)
Periocular	1	—*	—*	9.5 (3.5–12.6)	18.7 (6.3–21.7)

Route	Vitreous				Ratio (Vitreous/Plasma)	
	C_{max}, L, ng/mL	AUC, L, ng·h/mL	C_{max}, T, ng/mL	AUC, T, ng·h/mL	C_{max}, T	AUC, T
Animal model: rabbit						
Periocular	6.5 (2.0–9.5)	32.0 (4.6)	15.5 (9.5–19.5)	76.7 (7.5)	0.075	0.21
Intravenous	8 (5–13)	34.9 (4.0)	19 (17–23)	113.6 (6.7)	0.05	0.31
Intravitreal	4,550 (1,230–9,200)	6,560	5,300 (2,040–11,100)	26,620	254	56.6
Animal model: pig						
SSOAI	—*	—*	131.8 (112.9–138.7)	299.8 (247.6–347.2)	16.3	29
Periocular	—*	—*	13.6 (5.5–15.3)	48.9 (11.8–63.4)	1.5	3.4

*Only total topotecan was assayed.
C_{max}, maximal concentration; L, Lactone; T, total topotecan (carboxylate plus lactone).

added as a vasoconstrictor. When the implants were subconjunctivally implanted in rabbits, topotecan accumulated in the locally exposed sclera, choroid, and retina.[45] Plasma exposure was ~3 times lower than that obtained after the periocular injection of 1 mg of topotecan aqueous solution in rabbits.[42] A statistically significant increase in the vitreous concentration was achieved when topotecan was coloaded with adrenaline; however, this experimental formulation was not used in clinical practice. Concomitantly, Tsui et al[46] developed a drug-delivery system by loading topotecan into fibrin sealant, based on previous encouraging experience with carboplatin.[47] Different doses of topotecan were loaded into fibrin sealant (Tisseel, Baxter Healthcare), a biocompatible and biodegradable Food and Drug Administration–approved matrix used as a surgical adhesive indicated as an adjunct to hemostasis.[48] No pharmacokinetic determinations were done, and antitumor activity was evaluated in LHb-Tag transgenic mice. One of the advantages is that the delivery system is biodegradable as a consequence of biological fibrinolysis over time. Nevertheless, as seen with bolus injection, the hematogenous route also prevailed as the main absorption route, which was evidenced by significant tumor reduction in the contra-lateral control eyes.[46] Nonetheless, this formulation was translated to the clinic; Mallipatna et al[49] treated 10 eyes of 8 patients with topotecan in fibrin sealant given 1 to 4 times approximately every 3 weeks. All but three eyes had received previous local and/or systemic chemotherapy. Four of 10 patients (a total of 6 eyes: 2 Group D and 4 Group B) did not respond to treatment and required further systemic and/or local therapy. Tumor control was attained in four patients and in two patients in whom systemic chemotherapy was administered after a lack of tumor response. In addition, 11 and 10 cycles of 28 (39 and 36%, respectively) were followed by Grade 1 to 2 and Grade 3 to 4 hematologic toxicity, respectively, suggesting systemic absorption because some type of hematologic toxicity was recorded in 75% of the cycles. The local toxicity of topotecan in fibrin sealant seems to be low because ocular motility changes usually seen with carboplatin treatment were not found after this therapy.[50]

All together, these preclinical and clinical data suggest that most of the activity of periocular topotecan is related to systemic disposition,[34,42,49] and that selective transscleral passage of topotecan is poor. Therefore, this route of delivery for single agent therapy of retinoblastoma was abandoned by most groups in favor of other routes of administration with a better comparative pharmacokinetic profile.

Intravitreal Administration

Although traditionally contraindicated because of probable orbital tumoral seeding during injection, direct intravitreal chemotherapy administration for the treatment of retinoblastoma has recently received much attention after modifications of the injection

technique were proposed.[51] After direct injection, vitreous drug levels may be extremely high and maintained for longer periods of time depending on the dose and the mechanisms for posterior segment drug removal based on the lipophilicity and molecular size of the drug.[52] Topotecan is a candidate for this route of administration because it would allow for longer exposures favoring its activity as an S-phase agent. Topotecan's stability in diluted solutions is also an advantage for this route. Topotecan hydrochloride at a concentration of 0.05 mg/mL (equivalent to 50 μg/mL) in normal saline–sodium chloride 0.9% is chemically stable under room conditions (25°C) stored in plastic bags and infusion devices for 4 days.

Buitrago et al[53] reported on the pharmacokinetics of topotecan after a single and 4 weekly repeated intravitreal injection of 5 μg and 0.5 μg in the rabbit eye. After both 0.5 μg and 5 μg, the maximum concentration was attained after 5 minutes of drug injection. The attained vitreous levels were predictably high, in the micromolar range, exceeding potential pharmacologically active levels for ~16 hours and 5 hours after a single topotecan dose of 5 μg and 0.5 μg, respectively. Interestingly, it was shown that topotecan does not follow linear pharmacokinetics after intravitreal administration at higher doses. Topotecan elimination from the aqueous fluid was fast and became undetectable after 4 hours of administration. Thus, total topotecan AUC in the aqueous humor was only 5% of the vitreous exposure. Lastly, topotecan was present at all times in the aqueous humor as the carboxylate form probably as a result of a selective passage favoring this moiety over the lactone form or a possible greater affinity and retention of the lactone moiety to collagen fibers in the vitreous humor. Total topotecan systemic exposure was only 0.25% and 1.8% of the vitreous C_{max} and AUC of the injected eye, respectively. These data are supported by other groups who found very low levels of topotecan in plasma after a 1- and 2-μg dose.[54] After a 0.5- or 5-μg dose per week for 4 consecutive weeks, no accumulation was observed in the vitreous of the treated animals. The toxicity profile of intravitreal topotecan in nontumor-bearing rabbits was evaluated in detail in two studies from different groups.[54,55] Neither found toxicity at electroretinographic findings or hematopoietic changes. From the histopathologic point of view, Darsova et al[54] reported changes potentially attributable to toxicity in eyes treated with 1 and 2 μg of intravitreal topotecan. Nevertheless, these eyes were punctured for procurement of the vitreous samples, and thus it is not possible to rule out the effect of trauma in their results. However, Buitrago et al[55] failed to find any histopathologic evidence of toxicity in their cohort treated with 4 weekly intravitreal injections of up to 5 μg per dose of topotecan in a clinically relevant design specifically avoiding puncturing the eye. Topotecan has also been manufactured in nanoparticles[56,57] that would be potentially of use for intravitreal delivery; however, no experimental studies in patients have been reported as yet. Therefore, intravitreally administered topotecan at a dose of 5 μg achieved high vitreous levels without significant ocular or systemic toxicity, but its role in clinical practice remains to be determined.

Intraarterial Administration

Preclinical data on the ocular pharmacology of super-selective intraarterial (SSOAI) topotecan were based on experiments in a swine model.[58] After the administration of 1 mg of SSOAI topotecan infused over 30 minutes, topotecan achieved vitreous concentrations above its calculated IC50 till 4 hours showing a favorable ratio of vitreous-to-plasma exposure (Table 2). This study also compared the SSOAI with periocular administration of the same dose in the swine model. Although topotecan also reached the vitreous humor after periocular injection, this experiment confirmed its relatively low selectivity when administered by this route (Table 2). As presented in Table 2, vitreous C_{max} and AUC exposure parameters were significantly lower after periocular injection than after SSOAI while no significant difference was observed in systemic topotecan AUC when comparing between routes of administration. In the same nontumor-bearing swine model, the vitreous penetration

Table 2. Vitreous and Plasma Ratios of Pharmacokinetic Parameters According to Route in Different Animal Models

Route	C_{max} Ratio	AUC Ratio
Vitreous-to-plasma ratio for total topotecan		
Periocular (rabbit)	0.11	0.21
Periocular (pig)	1.4	3.4
Intravenous (rabbit)	0.07	0.31
Intra-vitreal (rabbit)	254	56.7
SSOAI (pig)	16.3	29
Vitreous-to-vitreous ratios for total topotecan		
Intravitreal/periocular (rabbit)	342	347
Intravitreal/intravenous (rabbit)	279	234
SSOAI/periocular (pig)	9.7	6
Plasma-to-plasma ratios for total topotecan		
Intravitreal/periocular (rabbit)	0.13	1.31
Intravitreal/intravenous (rabbit)	0.08	1.29
SSOAI/periocular (pig)	0.85	0.57

Intravitreal dose: 0.005 mg; periocular and intravenous: 1 mg.
C_{max}, maximal concentration.

Table 3. Comparative Ocular Pharmacokinetics Between Topotecan and Melphalan

Drug	Dose, mg	C_{max}, Vitreous, mM	AUC, Vitreous, μM·h	C_{max} Ratio (Vitreous/Plasma)	AUC Ratio (Vitreous/Plasma)	IC50 (nM)*
Topotecan	1 (2.2)	0.29	0.65	15.4	29	20–30
Melphalan	7 (22.9)	0.65	0.95	3.4	3.2	1,000–1,600

*Reported in Y79 and WERI-RB cell lines.
C_{max}, maximal concentration; IC, inhibitory concentration.

of topotecan compares favorably with that of melphalan, the most commonly used agent for SSOAI (Table 3).[32] Topotecan had a relative vitreous-to-plasma exposure at least five times higher than that of melphalan. In addition, pharmacologically active concentrations of topotecan in the vitreous of treated animals were attained until 16 hours postinfusion in contrast to the fast decay of melphalan by SSOAI. The high vitreous-to-plasma exposure ratio for topotecan may result from a favorable penetration of the drug through the blood-retinal barrier into the retina or a limited clearance of topotecan from the vitreous back to the systemic circulation. Indirect data suggest that topotecan administered by SSOAI may be less toxic than other drugs used by this route. Also, it has been associated to mild treatment-related changes in the electroretinographic findings in children,[30,59] and given its longer stability in diluted solutions, compared with melphalan, it may theoretically avoid the formation of toxic drug precipitates seen in a nonhuman primate model of melphalan.[60] The plasma pharmacokinetics of the association of intraarterial topotecan and melphalan has been recently reported.[61] Concomitant topotecan administration did not influence melphalan pharmacokinetic parameters, and there was no effect of the sequence of melphalan and topotecan administration in plasma pharmacokinetics.[61] However, the optimum dose of topotecan through this route remains to be established. Hence, topotecan is an interesting candidate drug for SSOAI based on the favorable penetration into or residence in the vitreous; however, its efficacy as a sole therapy when administered by this route is unknown although it has limited clinical value as a single agent. In addition, protracted exposure is not possible by this route, which may limit its clinical efficacy.

Conclusion and Future Directions

Topotecan is an active drug against retinoblastoma showing a favorable vitreous penetration and a safe toxicity profile by all routes of administration. Nevertheless, the periocular route is probably the least efficient in achieving high vitreous levels compared with other routes. The intravitreal route is very promising but it is still in the preclinical phase, and additional studies in tumor-bearing animal models may provide more information on schedule, efficacy, and toxicity with the final aim of adding another drug to the limited armamentarium for intravitreal chemotherapy virtually including only melphalan in clinical use. As done for SSOAI, preclinical studies comparing melphalan and topotecan intravitreally may provide additional information with potential clinical implications. Despite these favorable features, it is currently difficult to establish its role as a single agent for the treatment of retinoblastoma. Given its low ocular toxicity by all routes of administration, it is probable that the place of topotecan in the therapeutic armamentarium of retinoblastoma lies in multiagent chemotherapy regimens, probably combining more than one administration route.

Key words: topotecan, camptothecins, retinoblastoma, ocular pharmacokinetics, vitreous.

References

1. Canturk S, et al. Survival of retinoblastoma in less-developed countries impact of socioeconomic and health-related indicators. Br J Ophthalmol 2010;94:1432–1436.
2. Kingston JE, et al. Results of combined chemotherapy and radiotherapy for advanced intraocular retinoblastoma. Arch Ophthalmol 1996;114:1339–1343.
3. Shields CL, Shields JA. Retinoblastoma management: advances in enucleation, intravenous chemoreduction, and intra-arterial chemotherapy. Curr Opin Ophthalmol 2010;21:203–212.
4. Shields CL, et al. Chemoreduction for group E retinoblastoma: comparison of chemoreduction alone versus chemoreduction plus low-dose external radiotherapy in 76 eyes. Ophthalmology 2009;116:544–551 e1.
5. Lee SH, et al. Tandem high-dose chemotherapy and autologous stem cell rescue in children with bilateral advanced retinoblastoma. Bone Marrow Transplant 2008;42:385–391.
6. Gaudana R, et al. Recent perspectives in ocular drug delivery. Pharm Res 2009;26:1197–1216.
7. Doz F, et al. Etoposide and carboplatin in extraocular retinoblastoma: a study by the Societe Francaise d'Oncologie Pediatrique. J Clin Oncol 1995;13:902–909.
8. Chantada GL, et al. Phase II window of idarubicin in children with extraocular retinoblastoma. J Clin Oncol 1999;17:1847–1850.
9. Parareda A, et al. Intra-arterial chemotherapy for retinoblastoma. Challenges of a Prospective Study. Acta Ophthalmol 2013. In press.

10. Schouten-van Meeteren AY, et al. Histopathologic features of retinoblastoma and its relation with in vitro drug resistance measured by means of the MTT assay. Cancer 2001;92:2933–2940.
11. Barrie SE, et al. High-throughput screening for the identification of small-molecule inhibitors of retinoblastoma protein phosphorylation in cells. Anal Biochem 2003;320:66–74.
12. Hendig D, et al. Characterization of the ATP-binding cassette transporter gene expression profile in Y79: a retinoblastoma cell line. Mol Cell Biochem 2009;328:85–92.
13. Suarez F, et al. Paclitaxel in the treatment of retinal tumors of LH beta-Tag murine transgenic model of retinoblastoma. Invest Ophthalmol Vis Sci 2007;48:3437–3440.
14. Pina Y, et al. Retinoblastoma treatment: utilization of the glycolytic inhibitor, 2-deoxy-2-fluoro-D-glucose (2-FG), to target the chemoresistant hypoxic regions in LH(BETA)T(AG) retinal tumors. Invest Ophthalmol Vis Sci 2012;53:996–1002.
15. Dyer MA, Rodriguez-Galindo C, Wilson MW. Use of preclinical models to improve treatment of retinoblastoma. PLos Med 2005;2:e332.
16. Shields CL, et al. Factors predictive of recurrence of retinal tumors, vitreous seeds, and subretinal seeds following chemoreduction for retinoblastoma. Arch Ophthalmol 2002;120:460–464.
17. Shen J, et al. Compartment-specific roles of ATP-binding cassette transporters define differential topotecan distribution in brain parenchyma and cerebrospinal fluid. Cancer Res 2009;69:5885–5892.
18. Blaney SM, et al. Phase I clinical trial of intrathecal topotecan in patients with neoplastic meningitis. J Clin Oncol 2003;21:143–147.
19. Dimaras H, et al. Retinoblastoma CSF metastasis cured by multimodality chemotherapy without radiation. Ophthalmic Genet 2009;30:121–126.
20. Nitschke R, et al. Topotecan in pediatric patients with recurrent and progressive solid tumors: a Pediatric Oncology Group Phase II Study. J Pediatr Hematol Oncol 1998;20:315–318.
21. Frangoul H, et al. Phase I study of topotecan administered as a 21-day continous infusion in children with recurrent solid tumors: a report from the Children's Cancer Group. Clin Cancer Res 1999;5:3956–3962.
22. Chantada GL, et al. Activity of topotecan in retinoblastoma. Ophthalmic Genet 2004;25:37–43.
23. Laurie NA, et al. Topotecan combination chemotherapy in two new rodent models of retinoblastoma. Clin Cancer Res 2005; 11:7569–7578.
24. Athale UH, et al. Phase I study of combination topotecan and carboplatin in pediatric solid tumors. J Clin Oncol 2002;20:88–95.
25. Nemeth KM, et al. Subconjunctival carboplatin and systemic topotecan treatment in preclinical models of retinoblastoma. Cancer 2011;117:421–434.
26. Qaddoumi I, et al. Topotecan and vincristine combination is effective against advanced bilateral intraocular retinoblastoma and has manageable toxicity. Cancer 2012;118:5663–5670.
27. Panetta JC, et al. Using pharmacokinetic and pharmacodynamic modeling and simulation to evaluate importance of schedule in topotecan therapy for pediatric neuroblastoma. Clin Cancer Res 2008;14:318–325.
28. Santana VM, et al. A pilot study of protracted topotecan dosing using a pharmacokinetically guided dosing approach in children with solid tumors. Clin Cancer Res 2003;9:633–640.
29. Schaiquevich P, et al. Intra-arterial chemotherapy is more effective than sequential periocular and intravenous chemotherapy as salvage treatment for relapsed retinoblastoma. Pediatr Blood Cancer 2013;60:766–770.
30. Francis JH, et al. Carboplatin +/- topotecan ophthalmic artery chemosurgery for intraocular retinoblastoma. PLoS One 2013; 8:e72441.
31. Gobin YP, et al. Intra-arterial chemotherapy for the management of retinoblastoma: four-year experience. Arch Ophthalmol 2011;129:732–737.
32. Schaiquevich P, et al. Pharmacokinetic analysis of melphalan after superselective ophthalmic artery infusion in preclinical models and retinoblastoma patients. Invest Ophthalmol Vis Sci 2012;53:4205–4212.
33. Brennan RC, et al. Targeting the p53 pathway in retinoblastoma with subconjunctival Nutlin-3a. Cancer Res 2011;71: 4205–4213.
34. Pescosolido N, et al. Topotecan hydrochloride effects on retinal vessels in newborn rats. Histol Histopathol 2012;27:497–506.
35. Han S, Wei W. Camptothecin induces apoptosis of human retinoblastoma cells via activation of FOXO1. Curr Eye Res 2011;36:71–77.
36. Giuliano M, et al. Induction of apoptosis in human retinoblastoma cells by topoisomerase inhibitors. Invest Ophthalmol Vis Sci 1998;39:1300–1311.
37. Barot M, et al. In vitro moxifloxacin drug interaction with chemotherapeutics: implications for retinoblastoma management. Exp Eye Res 2014;118:61–71.
38. Dunkel IJ, et al. Intensive multimodality therapy for patients with stage 4a metastatic retinoblastoma. Pediatr Blood Cancer 2010;55:55–59.
39. Wilson TW, et al. Penetration of chemotherapy into vitreous is increased by cryotherapy and cyclosporine in rabbits. Arch Ophthalmol 1996;114:1390–1395.
40. Abramson DH. Periocular chemotherapy for retinoblastoma: success with problems? Arch Ophthalmol 2005;123:128–129; author reply 129.
41. Mendelsohn ME, et al. Intraocular concentrations of chemotherapeutic agents after systemic or local administration. Arch Ophthalmol 1998;116:1209–1212.
42. Carcaboso AM, et al. Topotecan vitreous levels after periocular or intravenous delivery in rabbits: an alternative for retinoblastoma chemotherapy. Invest Ophthalmol Vis Sci 2007;48:3761–3767.
43. Chantada GL, et al. A phase I study of periocular topotecan in children with intraocular retinoblastoma. Invest Ophthalmol Vis Sci 2009;50:1492–1496.
44. Pontes de Carvalho RA, et al. Delivery from episcleral exoplants. Invest Ophthalmol Vis Sci 2006;47:4532–4539.
45. Carcaboso AM, et al. Episcleral implants for topotecan delivery to the posterior segment of the eye. Invest Ophthalmol Vis Sci 2010;51:2126–2134.
46. Tsui JY, et al. Subconjunctival topotecan in fibrin sealant in the treatment of transgenic murine retinoblastoma. Invest Ophthalmol Vis Sci 2008;49:490–496.
47. Van Quill KR, et al. Subconjunctival carboplatin in fibrin sealant in the treatment of transgenic murine retinoblastoma. Ophthalmology 2005;112:1151–1158.
48. Spotnitz WD. Fibrin sealant: past, present, and future: a brief review. World J Surg 2010;34:632–634.
49. Mallipatna AC, et al. Periocular topotecan for intraocular retinoblastoma. Arch Ophthalmol 2011;129:738–745.
50. Yousef YA, et al. No ocular motility complications after subtenon topotecan with fibrin sealant for retinoblastoma. Can J Ophthalmol 2013;48:524–528.
51. Munier FL, et al. Intravitreal chemotherapy for vitreous disease in retinoblastoma revisited: from prohibition to conditional indications. Br J Ophthalmol 2012;96:1078–1083.
52. Edelhauser HF, et al. Ophthalmic drug delivery systems for the treatment of retinal diseases: basic research to clinical applications. Invest Ophthalmol Vis Sci 2010;51:5403–5420.

53. Buitrago E, et al. Pharmacokinetic analysis of topotecan after intra-vitreal injection. Implications for retinoblastoma treatment. Exp Eye Res 2010;91:9–14.
54. Darsova D, et al. Topotecan vitreous and plasma levels and retinal toxicity after transcorneal intravitreal delivery in healthy albino rabbits: alternative retinoblastoma treatment. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2012; 156:318–323.
55. Buitrago E, et al. Ocular and systemic toxicity of intravitreal topotecan in rabbits for potential treatment of retinoblastoma. Exp Eye Res 2013;108:103–109.
56. Souza LG, et al. Development of topotecan loaded lipid nanoparticles for chemical stabilization and prolonged release. Eur J Pharm Biopharm 2011;79:189–196.
57. Gary-Bobo M, et al. Multifunctionalized mesoporous silica nanoparticles for the in vitro treatment of retinoblastoma: drug delivery, one and two-photon photodynamic therapy. Int J Pharm 2012;432:99–104.
58. Schaiquevich P, et al. Pharmacokinetic analysis of topotecan after superselective ophthalmic artery infusion and periocular administration in a porcine model. Retina 2012;32: 387–395.
59. Francis JH, et al. Electroretinogram monitoring of dose-dependent toxicity after ophthalmic artery chemosurgery in retinoblastoma eyes: six year review. PLoS One 2014;9: e84247.
60. Tse BC, et al. Superselective intraophthalmic artery chemotherapy in a nonhuman primate model: histopathologic findings. JAMA Ophthalmol 2013;131:903–911.
61. Taich P, et al. Clinical pharmacokinetics of intra-arterial melphalan and topotecan combination in patients with retinoblastoma. Ophthalmology 2013. In press.