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Pharmacokinetic analysis of topotecan after intra-vitreal injection. Implications for retinoblastoma treatment

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ABSTRACT

Topotecan is a promising drug with activity against retinoblastoma, however, attaining therapeutic concentrations in the vitreous humor is still a challenge for the treatment of vitreous seeds in retinoblastoma. Our aim was to characterize topotecan pharmacokinetics in vitreous and aqueous humor, and to assess the systemic exposure after intra-vitreal injection in rabbits as an alternative route for maximizing local drug exposure. Anesthetized rabbits were administered intra-vitreal injections of 5 μg of topotecan. Vitreous, aqueous, and blood samples were collected at pre-defined time points. A validated high-performance liquid chromatography assay was used to quantitate topotecan (lactone and carboxylate) concentrations. Topotecan pharmacokinetic parameters were determined in vitreous, aqueous and plasma using a compartmental analysis.

Topotecan lactone concentrations in the vitreous of the injected eye were about 8 ng/mL 48 h after drug administration. The median maximum vitreous, aqueous and plasma total topotecan concentrations (C_{max}) were 5.3, 0.68 and 0.21 μg/mL, respectively. The C_{max} vitreous/aqueous of treated eyes and the C_{max} vitreous/plasma were approximately 8 and 254, respectively. Total topotecan exposure (AUC) in the vitreous of the injected eye was 50 times greater than the total systemic exposure. These findings suggest that intra-vitreal administration of only 5 μg of topotecan reaches significant local levels over an extended period of time while minimizing systemic exposure in the rabbit. Intra-vitreal topotecan administration offers a promising alternative route for enhanced drug exposure in the vitreous humor with potential application for treatment of vitreal seeds in retinoblastoma while avoiding systemic toxicities.

1. Introduction

Systemic chemoreduction using carboplatin-based regimens followed by local therapies is the current standard of care for the conservative treatment of intraocular retinoblastoma. This therapy is efficacious for the treatment of small and medium size tumors limited to the retina, however it is less successful for the treatment of advanced disease, especially when vitreous seeding of the tumor is evident. In these cases, the unfavorable prognosis for eye-preservation could be related to the difficulty for chemotherapeutic agents in reaching the avascular vitreous humor (Rodriguez-Galindo et al., 2007). The blood-retinal barrier hinders intraocular penetration of chemotherapy. Therefore achieving therapeutic concentrations of drugs in the vitreous humor via the systemic circulation remains a challenge (Cunha-Vaz, 2004; Lee and Robinson, 2001; Raghava et al., 2004; Ranta and Urtti, 2006; Wilson et al., 1996).

In order to improve the vitreous drug delivery while attaining low systemic toxicity, alternative routes to systemic drug administration are under investigation. In children, periocular administration of carboplatin has shown some anti-tumor activity against vitreous seeds. Despite being almost devoid of systemic toxicity, severe local toxicity including orbital fibrosis and atrophy of the optic nerve have been reported (Abramson et al., 1999a, 1999b; Abramson, 2005; Schmack et al., 2006).

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We previously studied the pharmacokinetics of periocular topotecan given its promising anti-tumor activity against xenografts derived from childhood solid tumors and patients (Houghton et al., 1995; Nitschke et al., 1998; Laurie et al., 2005; Chantada et al., 2004). After periocular administration, topotecan lactone levels were attained at potentially active concentrations in the rabbit vitreous humor (Carcaboso et al., 2007). Results from a recent Phase I study in relapsed-refractory retinoblastoma also demonstrated that periocular topotecan was associated with mild local toxicity and relative low systemic exposure when comparing to the i.v. administration (Chantada et al., 2009). However, a rapid orbital clearance to the systemic circulation was found in our animal model.

Another alternative for achieving higher drug levels in the vitreous is directly injecting the drug into the vitreous body. For retinoblastoma, intra-vitreal injection of thiotepa and melphalan have been preliminarily studied (Kaneko and Suzuki, 2003). However, concerns of tumor seeding the orbit during drug intravitreal administration limited the widespread use of this technique. Nevertheless, recent improvements in the technique of intra-vitreal injection and the increased ability.to determine the location of tumor, thus avoiding entering over those regions during injection when it is feasible; suggest that this technique could be reconsidered as a feasible alternative for systemic chemotherapy.

Therefore, the aim of this work was to study the ocular pharmacokinetics of topotecan after intra-vitreal administration in an animal model with the aim of establishing its potential for treatment of retinoblastoma in the presence of vitreous seeds.

2. Materials and methods

2.1. Animal studies

Forty six New Zealand White rabbits (weighing 1.8–2.2 kg) were anesthetized with ketamine (37.5 mg/kg, IM) and xylazine (5 mg/kg, IM). The present work adheres to the tenets of Association for Research in Vision and Ophthalmology for the use of animals in ophthalmic and vision research. Only one eye of each animal received an intra-vitreal injection of topotecan. The fellow eyes were used as controls. The injection was performed with a 32G needle through the pars plana. For the pharmacokinetic study the dose was defined as 5 μg per animal.

2.2. Topotecan administration and sampling schedule

Topotecan solution (50 μg/mL, Hycamtin®) was prepared in 0.9% saline solution and 0.1 ml was injected using a 32 G needle coupled to a Hamilton syringe. Vitreous humor (100 μL) samples were obtained in the anesthetized animal by aspiration from the inner region of posterior ipsilateral eye chamber with a 18 G needle inserted in the superior region of the sclera approximately 3 mm from the limbus Aqueous humor samples (100 μL) were obtained by aspiration using a 18 G needle. Vitreous humor samples were obtained at: 0.083, 0.25, 0.75, 1.5, 4, 8, 16 and 48 h with concomitant aqueous humor sampling only up to 4 h post-injection as no measurable topotecan was found at longer times. Early time points were collected from animals that remained anaesthetized after ketamine/xylazine administration for topotecan administration. In order to obtain later samples (from 4 to 48 h post-administration), the animals we re-anaesthetized using the same procedure as previously described for topotecan administration and thereafter, the vitreous sample was obtained. Only one sample was collected from each eye in order to avoid modifying ocular physiology or topotecan disposition. All samples were collected and placed into an eppendorf, vortex mixed for 10 s. Then, 50 μL were transferred into a tube containing 200 μL of cold methanol to precipitate the proteins and stabilize topotecan equilibrium between the lactone and the carboxylate form. Venous blood samples (1 mL) were collected from the ear vein in heparinized tubes at: 0.083, 0.25, 0.75, 1.5, 4, 8, 16, 24, 44 and 48 h after topotecan intra-vitreal administration. Plasma samples were treated as previously described and all methanolic supernatant extracts were isolated and stored at −20 °C until topotecan analysis.

After collecting the samples, the animals were euthanatized with a rapid intra-cardiac bolus injection of 100 mg of sodium thiopental.

2.3. Topotecan measurement

Topotecan undergoes a pH-dependent reversible hydrolysis from the lactone that is the main pharmacologically active moiety to the carboxylate form that predominates at low pH values (Herben et al., 1996). Thus, it is important to quantitate lactone and total topotecan (lactone plus carboxylate). Topotecan lactone and carboxyate concentrations were determined using a modified HPLC method previously reported by others and validated by our group (Warner and Burke, 1997).

Appropriate dilutions of stock solution were made in phosphate buffers pH 3 or pH 10 to obtain the respective calibration curve for vitreous and aqueous topotecan lactone and carboxylate sample analysis. The lower limit of quantitation was set at 1 ng/mL, the within-day and between-day precision was less than 7%.

2.4. Pharmacokinetic study

The model building was performed sequentially and the lactone form and total (lactone plus carboxylate) were considered separately in the analysis.

A three-compartment model was fit to total topotecan vitreous, aqueous and plasma concentration-time data from all the animals using the maximum likelihood estimation method as implemented in ADAPT II (D'Argenio and Schumitzky, 2006). Model parameters that were estimated include the inter-compartmental rate constants of transfer of total topotecan from: the vitreous to the aqueous (Kva), the aqueous to the plasma (Kap), the vitreous to the plasma (Kvp), the plasma to the vitreous (Kpv) and the elimination from the plasma compartment (Kpl) respectively. In all cases, first order transfer rates were assumed. The absorption compartment was modeled as the vitreous compartment and from there, distributed to the aqueous and plasma compartments, respectively.

A particular behavior was observed in the data of the aqueous compartment. Total topotecan absorption was followed by a very fast elimination from the aqueous compartment with no detectable levels after 4 h of drug intra-vitreal injection. Different approaches were hypothesized so as to model the pharmacokinetic behavior of the drug. Thus, the rate of transfer between the vitreous to the aqueous humor was modeled as Kva1 until the maximum aqueous topotecan concentration and thereafter, Kva2. However, as aqueous humor levels were based on 3 time point determinations due to its rapid clearance and no data of *in vitro* diffusion of topotecan from the vitreous gel was available, this temporal variation could only be set as a discrete value. The constant rate of transfer between the aqueous to the plasma compartment (Kap) was fixed at the value obtained when individually modeling the aqueous concentration-*versus*-time data.

Total vitreous, aqueous, and plasma topotecan concentration-*versus*-time data from all the animals were simultaneously fit and the parameters estimated were used to simulate the plasma concentration-*versus*-time curve from which the area under the curve up to the last measurable time point (AUC) was calculated by use of the trapezoidal method.

3. Results

A total of 28, 12 and 27 samples of vitreous, aqueous and plasma, respectively from 46 animals were obtained in this study for the pharmacokinetic analysis. Aqueous samples were only collected up to 4 h after topotecan injection due to its fast clearance from the aqueous compartment. Thus, as no detectable concentrations were observed after 4 h of drug injection in three different animals it was decided not to obtain later samples.

The disposition of total topotecan and lactone in the vitreous humor of the injected eye (5 μg per animal) was well-described by a two-compartment model as represented in Fig. 1. The observed median maximum concentration (C_{max}) attained in the vitreous as lactone and total topotecan was 4.55 μg/mL (range, 1.23–9.2 μg/mL) and 5.3 μg/mL (range, 2.04–11.10 μg/mL), respectively. The time to maximum concentration T_{max} was observed in both cases after 5 min of drug injection. Low levels of topotecan were also detected in the vitreous humor of the contralateral eye in similar concentrations as those found in the plasma (data not shown).

An important result was that topotecan lactone AUC corresponded to approximately 25% of the total topotecan exposure in the vitreous compartment (Table 1). Moreover, detectable topotecan lactone levels (median, 8.1 ng/mL) were still observed up to 48 h after drug administration as shown in Fig. 2.

A three-compartment model was fit to the data obtained from vitreous, aqueous and plasma simultaneously. The model adequately described the experimental data as presented in Fig. 3. The pharmacokinetic parameter estimates obtained are reported in Table 2. We observed a rapid topotecan diffusion from the vitreous to the aqueous compartment followed by a considerably fast disappearance from the latter compartment. This rapid transfer rate from the vitreous compartment was modeled as Kva1 and changed over time to Kva2. In addition, only topotecan carboxylate was detected in the aqueous compartment up to 1.5 h after drug injection; after that no drug was detected in this compartment. Thus, only 5% of the drug exposure was observed in the aqueous with respect to the vitreous compartment. The model-predicted maximum concentration (C_{max}) in the aqueous compartment was 1.44 μg/mL attained after approximately 30 min of topotecan injection.

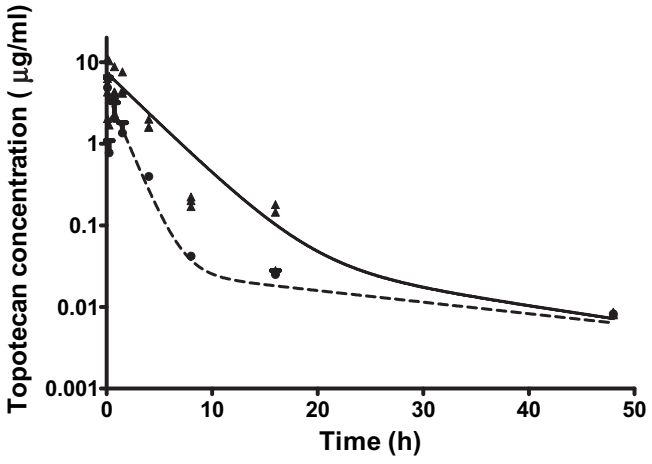

Fig. 1. Total (▲) and lactone (●) topotecan concentration-*versus*-time profile in vitreous humor after intra-vitreal injection (5 μg) in rabbits. Symbols represent individual data points and lines represent the predicted concentrations for total (solid) and topotecan lactone (dashed) in vitreous humor (n = 3–5 per time point). The inset shows the same concentration-*versus*-time profile with an expanded time scale up to 4 h after topotecan administration.

Table 1
Total topotecan and lactone drug exposure measured as area under the curve and maximum concentration in vitreous humor, aqueous humor and plasma.

Pharmacokinetic parameter	AUC_T (μg h/ml)	%AUC[a]	AUC_L (μg h/ml)
Vitreous (injected eye)	26.62	___	6.56[a]
Aqueous	1.35	5.1	___[b]
Plasma	0.469	1.8	___

Abbreviations: AUC_T, total topotecan area under the concentration-*versus*-time profile; AUC_L, topotecan lactone area under the concentration-*versus*-time profile.
[a] Percentage of total topotecan exposure (AUC) in the aqueous or plasma with respect to the vitreous compartment.
[b] Only the carboxylate form of topotecan was present in the aqueous humor.

A main concern about the administration of chemotherapy is the systemic exposure due to the related toxicity often encountered. However, an encouraging result obtained in the present study, as shown in Table 1, is that topotecan systemic exposure (AUC_T) calculated in plasma was only 1.8% of the vitreous AUC_T In addition, taking into account C_{max} as another parameter of drug exposure, we observed that total topotecan C_{max} vitreous/plasma was 254. This finding corroborates the selectivity of the intravitreal route of injection. No detectable topotecan was found in the plasma until 45 min after the drug administration. This could be related with a delay in the absorption process from the vitreous to the plasma through the adjacent choroid that plays a role as a possible mechanism of drug transport back to the vitreous compartment. Furthermore, model-predicted total C_{max} topotecan in plasma was 17.5 ng/mL with a T_{max} of 7.6 h.

4. Discussion

In the present study we showed that high topotecan concentrations were attained in the vitreous humor of the injected eye after the direct intra-vitreal administration of a low dose of the drug while achieving low systemic exposure.

Intra-vitreal administration of drugs is effective in the treatment of several vitreo-retinal diseases but there are few reports about the

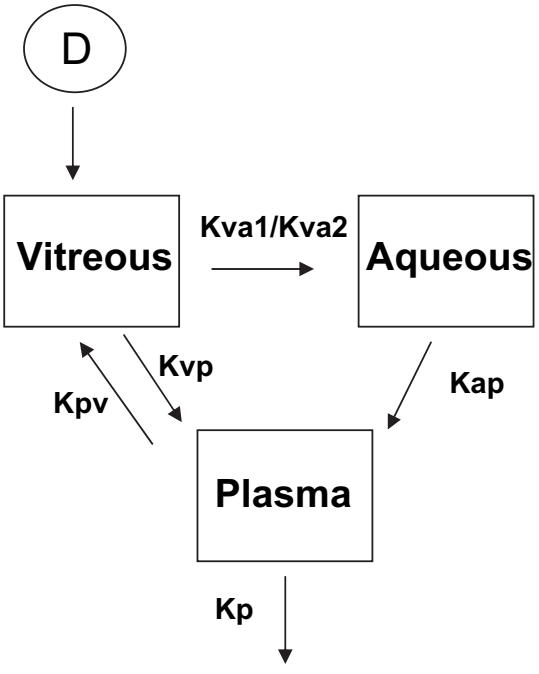

Fig. 2. Three-compartment model pharmacokinetic model for vitreous, aqueous and plasma total topotecan modeling.

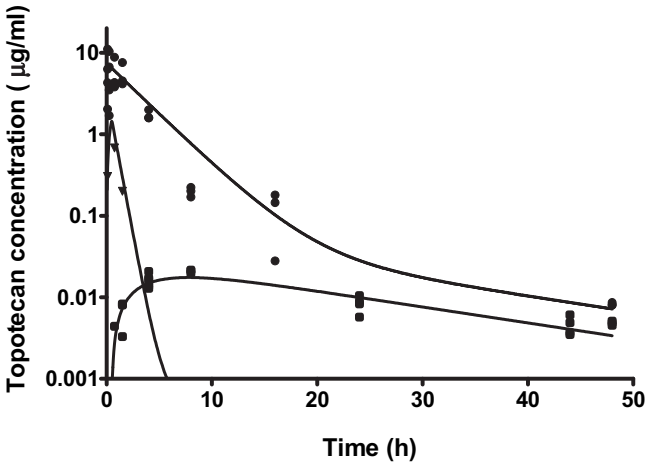

Fig. 3. Total topotecan vitreous (●), aqueous (▲) and plasma (■) concentration-*versus*-time profile after intra-vitreal injection. Symbols represent individual data points and lines represent the best-predicted concentrations for topotecan ($n = 3–5$ per time point for vitreous, aqueous and plasma data). Aqueous levels at 4 h were below the detection limit, so they are not represented in the figure.

characterization of the ocular pharmacokinetics of drugs using this route of administration (Bakri et al., 2007; Ficker et al., 1990; Hosseini et al., 2008; Kamppeter et al., 2008; Kim et al., 2006).

To our knowledge, there is no previously reported information about topotecan pharmacokinetics when the drug is administered directly into the vitreous. This information is particularly useful when considering alternative routes and drugs for the treatment of retinoblastoma with vitreous seeds. However pharmacokinetic results obtained in animals extrapolated to the clinical setting may not be completely adequate due to differences in the anatomy and physiology of the eye of different species. Another factor to consider is the hypothesis that a tumor in the retina may disrupt the blood-retina barrier thus modifying the clearance of the drug from the vitreous humor to the systemic circulation (Cunha-Vaz, 2004; Wilson et al., 1996). Given the limitations of any animal model, the pharmacokinetic study of topotecan disposition in the rabbit eye should be considered an approximation that allows an approach to chemotherapy of retinoblastoma, especially for those with vitreous seeds.

First, we investigated the dose of topotecan that would be suitable for intra-vitreal injection in the rabbit. Previous reports on intra-vitreal administration of melphalan showed that an effective clinical dose of 10 μg would yield an intra-vitreal concentration of about 5.9 μg/mL in rabbits (Kaneko and Suzuki, 2003). In addition, melphalan is administered for other malignancies at a systemic dose of 16 mg/m2. Thus, assuming an average body surface area of approximately 1 m^2, the intra-vitreal dosage of melphalan for retinoblastoma treatment is 3200 times less than that for the systemic therapy. In concordance, topotecan is systemically administered for the treatment of a variety of pediatric tumors including retinoblastoma on a protracted schedule of five consecutive days each week for two weeks at a dosage of 2 mg/m^2/day. Hence, if maintaining the same relationship as for melphalan, between systemic and intra-vitreal dosage, the theoretical topotecan dose to be administered directly into the eye would fall between 5 and 7 μg/animal. Thus, we initially studied three dose levels of 5, 50 and 200 μg of intra-vitreal topotecan. The studied levels yielded total topotecan concentration in the vitreous of 5.5, 31.0, and 211.5 μg/mL, respectively after 0.5 h of the drug injection. Considering the preliminary results, we decided to perform the pharmacokinetic study on the 5 μg dose per animal.

In a previous study, we reported that 1 mg of topotecan administrated periocularly to rabbits yielded total topotecan vitreous humor exposure (AUC_T) of 76,7 ng h/mL and a lactone vitreous exposure (AUC_L) of 32.0 ng h/mL (Carcaboso et al., 2007). In the present study topotecan AUC_T and AUC_L in the vitreous humor was 26 620 and 6560 ng h/mL respectively after the intra-vitreal administration of 5 μg of drug (Table 1). This indicates that total vitreous exposure after the intra-vitreal injection was almost 347 times higher than after the periocular administration when dosing 200 times less amount of drug directly into the eye. Considering the lactone moiety of topotecan, the ratio for vitreous exposure obtained after intra-vitreal injection with respect to the periocular route was about 205:1. Moreover, the ratio between the median maximum vitreous total topotecan concentration after the intra-vitreal injection with respect to the periocular administration was 360:1 (14,6 ng/mL versus 5300 ng/mL) demonstrating an enormous increase in the vitreous drug exposure by directly administering the drug into it. Laurie et al. have previously shown that 15 min of topotecan exposure *in vitro* was enough to achieve the maximum reduction in cell survival and proliferation at the LC50 concentration (concentration required to reduce cell viability by 50%) (Laurie et al., 2005). This concentration was reported to be 30 nM or equivalent to 14 ng/mL Thus, we can hypothesize that after the intra-vitreal injection of 5 μg of topotecan, we attained potentially cytotoxic concentrations in vitreous humor up to 16 h after drug administration. However, this is only an approximation due to the fact that previous reports were obtained *in vitro* from Y79 cell culture and we are reporting data from topotecan disposition in an animal model.

The concentration in the vitreous body is not homogeneous and the area near the injection site will be exposed to higher local concentrations than the rest of the vitreous until drug diffusion occurs (Tojo, 2004). Therefore some of our data, especially from the earlier time points show wide variability. However, it would be considerably time consuming to carry out a pharmacokinetic study that takes into account local drug concentrations in different areas of the vitreous. Another alternative would be to enucleate the eye, surgically remove and homogenize the vitreous before topotecan quantitation. However, it would not be accurate for drugs that undergo time-dependant conversion to inactive forms like topotecan. Therefore, in the present study we decided to obtain vitreous humor by aspiration which enabled us to collect samples at different times after drug administration and immediately precipitate with cold methanol to stabilize the lactone form.

The elimination half-life of a drug from the vitreal cavity depends on two pathways, the anterior route into the aqueous and

Table 2
Pharmacokinetic parameter estimates for total topotecan.

Pharmacokinetic parameter	Estimate	CV%
Kva1 (h^{-1})	0.180	13.3
Kvp (h^{-1})	0.287	6.0
Kpv (h^{-1})	0.0018	31.1
Kpl (h^{-1})	0.045	10.4
Vpl/F (ml)	202.2	11.1

Abbreviations: Kva1, vitreous to aqueous compartment microrate constant value (from vitreous injection until maximum aqueous concentration, C_{max}); Kap, aqueous to plasma compartment microrate constant; Kvp, vitreous to plasma compartment microrate constant; Kpv, plasma to vitreous compartment microrate constant; Kpl, elimination rate constant from the plasma compartment; Vpl/F, apparent volume of distribution in the plasma compartment.
The volume of distribution of the vitreous and the aqueous compartments was fixed to 0.7 mL and 0.2 mL, respectively. The aqueous to plasma (Kap) and vitreous to aqueous (Kav2) after the C_{max} in the aqueous compartment microrate constants were fixed to 1.6 h^{-1} and 0.00017 h^{-1}, respectively. The time for conversion between Kva1 and Kva2 was fixed to 30 min.

the posterior route by active transport across the retina (Mannermaa et al., 2006; Raghava et al., 2004; Ranta and Urtti, 2006). In the present study, topotecan diffusion from the vitreous to the aqueous humor was very fast with a maximum vitreous concentration attained after 45 min of drug administration. However, topotecan disappearance from the aqueous compartment was also fast, and the drug was below the limit of quantitation after 4 h of drug administration. Thus, total topotecan exposure in the aqueous humor was only 5% of the AUC_T calculated from the vitreous data. One interesting and unexpected finding is that no detectable topotecan lactone was found in the aqueous humor at any time. This phenomenon may be partly explained by a more selective passage of the carboxylate form to the aqueous chamber, or a possible greater affinity of the lactone moiety to proteins in the vitreous humor. The vitreous is composed of macromolecules such as collagen fibers that may alter the equilibrium between the lactone and carboxylate forms potentially favoring the latter form that is available for diffusion to the aqueous chamber (Mi and Burke, 1994). Studies of the equilibrium between the two forms of topotecan in a medium similar to vitreous should be performed to support this hypothesis.

Besides being cleared by the anterior chamber, topotecan can also traverse the blood-retinal barrier into the systemic circulation. This route of transport involves permeation of the drug through the different ocular tissue layers, and may also be subject to drug transporters localized in the blood-retinal barrier even at the level of the retinal pigment epithelium (Hosoya et al., 2009; Mannermaa et al., 2009). In accordance to this assumption, we observed a delay or lag-time in topotecan drug permeation to the plasma for about 45 min after drug administration. Maximum plasma concentration (C_{max}), which could be considered a surrogate of the diffusion rate, was achieved after 8 h of drug administration and was only 0.25% of the vitreous $C_{max.}$ In addition, topotecan systemic drug exposure was only 1.8% of the vitreous AUC_T obtained in the injected eye. This low C_{max} attained in the plasma compartment corresponds to the low permeation of topotecan from the vitreous humor and is also due to distribution through a systemic volume of about 202 mL. Furthermore, systemic exposure in children, given this same dose, would be even less because the volume of distribution is larger than that of rabbits while the vitreous volume is comparable between species.

In previous studies we showed a rapid distribution to plasma and elimination from it after periocular administration of topotecan (Carcaboso et al., 2007; Chantada et al., 2009). Our results showed that the relationship between the median total plasma topotecan C_{max} after intra-vitreal and periocular administration in rabbits was 0.14. Thus, after the intra-vitreal administration the drug reaches the systemic circulation after a lag-time in a sustained fashion and lower levels are attained in contrast to the periocular route.

Therefore intra-vitreal administration yields high vitreous concentrations of chemotherapy and minimizes systemic exposure. Even though this would be an advantage for this route of administration of chemotherapy, its safety must be considered before translating these findings to the clinical setting. Intravitreal administration has been considered to increase the risk of extraocular extension of the tumor due to the introduction of the needle and disruption of ocular boundaries. Moreover, repeated intra-vitreal administration can lead to retinal detachment and, when considered in the clinical setting, surgical removal of the vitreous will be likely after several treatments. In addition, as high local concentration of drugs is achieved, potential retinal toxicity may occur. Therefore, further experiments assessing ocular toxicity after topotecan given through various routes of administration are under evaluation.

Another alternative for local administration of topotecan for its use in retinoblastoma could be its administration through a selective ophthalmic artery infusion of chemotherapy as was reported for melphalan (Abramson et al., 2008). However, no pharmacokinetic data are available at this time.

Topotecan is a S-phase specific antineoplastic drug, and previous preclinical and clinical studies shown that its anti-tumor activity is more favorable with protracted schedules (Panetta et al., 2008; Santana et al., 2003)..One possible additional advantage for local topotecan administration is the potential synergism in the pharmacological response when administered with systemic carboplatin. These drugs together resulted in the most active combination *in vitro* and *in vivo* (Laurie et al., 2005).

Hematopoietic toxicity limits the concomitant i.v administration of both agents however, chemotherapy combination would be a better alternative for retinoblastoma treatment, justifying the development of innovative drug delivery modalities.

In summary, we show in the present report that high and potentially therapeutic concentrations were attained in the vitreous humor of the injected eye after intra-vitreal administration of topotecan up to 48 h after drug administration with low systemic exposure. Therefore, this administration modality should be further investigated as an approach to treating advanced retinoblastoma in the presence of vitreous seeding.

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