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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.