Clinical Pharmacokinetics of Intra-arterial Melphalan and Topotecan Combination in Patients with Retinoblastoma

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Purpose: To assess the antitumor activity, toxicity, and plasma pharmacokinetics of the combination of melphalan and topotecan for superselective ophthalmic artery infusion (SSOAI) treatment of children with retinoblastoma.

Design: Single-center, prospective, clinical pharmacokinetic study.

Participants: Twenty-six patients (27 eyes) with intraocular retinoblastoma.

Methods: Patients with an indication for SSOAI received melphalan (3−6 mg) and topotecan (0.5−1 mg; doses calculated by age and weight). Plasma samples were obtained for pharmacokinetic studies, and a population approach via nonlinear mixed effects modeling was used. Safety and efficacy were assessed and compared with historical cohorts of patients treated with melphalan single-agent SSOAI.

Main Outcome Measures: Melphalan and topotecan pharmacokinetic parameters and efficacy and safety parameters.

Results: Twenty-seven eyes from 26 consecutive patients received 66 cycles of SSOAI melphalan and topotecan in combination. All 5 eyes treated as primary therapy responded to the combination chemotherapy and were preserved. Sixteen of the 22 eyes with relapsed or resistant tumors responded, but 3 of them ultimately underwent enucleation at a median of 8 months (range, 7.9−9.1 months). The incidence of grade III and IV neutropenia was 10.6% and 1.5%, respectively, which was comparable with historical controls of single-agent SSOAI melphalan. No episode of fever neutropenia was observed, and no patient required transfusion of blood products. The large variability in melphalan pharmacokinetics was explained by body weight ($P < 0.05$). Concomitant topotecan administration did not influence melphalan pharmacokinetic parameters. There was no effect of the sequence of melphalan and topotecan administration in plasma pharmacokinetics.

Conclusions: A regimen combining melphalan and topotecan for SSOAI treatment of retinoblastoma is active and well tolerated. This combination chemotherapy previously showed synergistic pharmacologic activity, and we herein provide evidence of not increasing the hematologic toxicity compared with single-agent melphalan. *Ophthalmology 2014;■:1−9 © 2014 by the American Academy of Ophthalmology.*

Melphalan monotherapy is the most common treatment administered by superselective ophthalmic artery infusion (SSOAI) for intraocular retinoblastoma.[1,2] The initial phase 1−2 study of SSOAI melphalan reported an 82% response rate in chemotherapy-naïve eyes,[3] and subsequent experience showed that the results in pretreated eyes were less encouraging.[4,5] Hence, some groups developed different strategies to improve the results in these high-risk eyes. One alternative is to use a higher dose of melphalan.[5] Unfortunately, doses higher than 0.5 mg/kg lead to increased risk of hematopoietic toxicity.[4,6] This is especially relevant when tandem therapy for the treatment of bilaterally affected children is necessary because the total dose should be divided in 2.[7,8] Another alternative would be to use combination chemotherapy without overlapping toxicity, as proposed by a New York group, with the combination of carboplatin, topotecan, and melphalan.[4,9] In this line, our group reported a synergistic effect of the combination of topotecan and melphalan in retinoblastoma cell lines that we speculated may be translated to the clinic in terms of increased activity.[6] This synergistic effect between a topoisomerase I inhibitor and an alkylating agent also was reported in other pediatric neuroectodermal malignancies.[10] Topotecan is an active drug for retinoblastoma with low systemic and ocular toxicity and well-established pharmacologic activity.[11,12] In addition, we previously showed a favorable pharmacokinetic profile of topotecan after SSOAI in the swine model, allowing a high passage into the vitreous and sustained concentrations compared with melphalan.[13] Thus, the addition of topotecan to melphalan would be an attractive option to enhance their pharmacologic activity, especially for targeting the vitreous of the affected eye. However, no information was available about the toxicity of that combination in patients with retinoblastoma.

Potential pharmacologic interactions between melphalan and topotecan may occur, and this should be addressed by studying

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the pharmacokinetics of these drugs. For instance, a pharmacokinetic drug−drug interaction could result at the renal secretion process of both drugs. Specifically, topotecan primarily is eliminated through the kidneys. Zamboni et al[14] reported that probenecid inhibited renal tubular secretion of topotecan, decreasing renal topotecan clearance and thus increasing the systemic exposure in the animal model. Taking into account that melphalan elimination is mainly through renal excretion and that in children it previously was reported to have a total clearance value of more than the normal creatinine clearance in pediatric patients, renal secretion of melphalan or its degradation products may not be disregarded.[15] Hence, a potential drug−drug interaction at the kidneys may result in increased systemic exposure of melphalan, yielding more than expected hematologic toxicity. In addition, drug−drug pharmacodynamic interactions should be evaluated because both drugs may cause severe neutropenia, and a potential synergistic effect could result in pronounced hematologic toxicity.[15,16]

Additionally, the sequence of drug administration may influence the pharmacokinetics of the infused drugs.[17,18] Specifically, SSOAI of melphalan could result in vascular effects that may alter the disposition of a subsequently administered drug.[19] Thus, a better understanding of the toxicity of melphalan and topotecan in combination after SSOAI and the sequence effect should be available for routine clinical practice.

Therefore, the aims of this study were to characterize the pharmacokinetics, toxicity, and antitumor activity of a combination of melphalan and topotecan administered by SSOAI to children with high-risk intraocular retinoblastoma.

Methods

Patients and Treatment

This study followed the tenets of the Declaration of Helsinki, and institutional review board approval was obtained. Written informed consent was obtained from the participants' parents or guardians. From October 2011 through July 2013, the melphalan−topotecan combination regimen was offered to all children with intraocular retinoblastoma and the following features:

1. Chemotherapy-naïve unilateral retinoblastoma for which eye-sparing therapy was proposed.
2. Group D eyes who were too young to receive SSOAI upfront and hence received 2 to 3 cycles of carboplatin-etoposide-vincristine systemic combination (bridge therapy[20]) before SSOAI. A minimum of 1 month elapsed from the last intravenous chemotherapy cycle and SSOAI was required.
3. Bilateral retinoblastoma either with relapsed or refractory intraocular disease after chemoreduction with systemic chemotherapy or after SSOAI chemotherapy with carboplatin-based regimens, melphalan as a single agent, or both, regardless of the intraocular grouping.

The SSOAI therapy was performed according to published guidelines.[3] Each drug was infused in a pulsatile fashion over 15 minutes and was administered sequentially in no predetermined order. Hence, the sequence of melphalan−topotecan and topotecan−melphalan was administered indistinctly in each cycle. The chemotherapy regimen included (1) melphalan administered at doses that previously were reported as 3 mg for children younger than 2 years, 4 mg for children from 2 to 3 years of age, and 5 mg for children older than 3 years,[4] with the maximum dosage of melphalan being 0.48 mg/kg; and (2) topotecan at a dose of 0.5 mg for those younger than 1 year and 1 mg for older children.[4] Melphalan was reconstituted bedside during the procedure, whereas topotecan was reconstituted in advance at the hospital pharmacy.[16]

Patients were examined clinically and were discharged 8 to 12 hours after the procedure. A complete blood cell count was performed routinely at 10 and 21 days after SSOAI or if required for clinical control.

Efficacy and Adverse Events Assessment

In this analysis, response was defined as tumor or seeds regression, or both, based on previously reported criteria.[21] The follow-up of each treated eye was assessed as the interval of time since the first cycle of melphalan and topotecan SSOAI until July 2013 or enucleation if it was required. Toxicity was graded according to the Common Terminology Criteria for Adverse Events version 4.0.[22]

Pharmacokinetic Studies: Sample Collection and Bioanalysis

Plasma pharmacokinetic studies were attempted for every cycle in which the patient received melphalan and topotecan combination, depending on the availability of a suitable peripheral intravenous access. One milliliter of blood was collected in heparinized tubes before starting chemotherapy administration and at the end of the infusion of each drug and 0.5, 1.0, 2.0, and 3.0 hours after finishing the infusion of the second drug.

Blood samples were centrifuged and plasma was separated and precipitated with cold acidic methanol as previously described. Methanolic supernatants were stored at −20° C until assayed. Melphalan and topotecan were assayed using a validated high-performance liquid chromatography method coupled with a fluorescence detector. The linear range for plasma melphalan and topotecan was from 10 to 700 ng/ml and from 5 to 150 ng/ml, respectively. The lower limit of quantitation of melphalan and topotecan was 50 and 5 ng/ml, respectively. The interday precision of melphalan and topotecan bioanalysis was less than 11% and 7%, respectively.

Pharmacokinetic Studies: Data Analysis

The pharmacokinetics of melphalan and topotecan were determined by means of a nonlinear mixed effects modeling method implemented in Monolix version (Modèles NOn Linéaires à effets mixtes; Incuballiance, Orsay, France). We tested both 1- and 2-compartment models to describe separately melphalan and topotecan plasma concentration-versus-time data. Different residual error models were evaluated, including a constant (additive) or mixed proportional and additive error model. Models were chosen based on the objective function value and visual inspection of goodness-of-fit diagnostic plots.

The relationship between individual patient-specific characteristics (covariates) and the pharmacokinetic parameters of melphalan or topotecan was evaluated by means of a univariate analysis. The covariates included dosage, age, sex, body weight, body surface area, and sequence of treatment (melphalan then topotecan or topotecan then melphalan). To retain a covariate in the pharmacokinetic model, we evaluated the significance to explain to some extent the interindividual variability observed in the parameters. A covariate was considered significant if its addition to the base model reduced the objective function value by at least 3.84 units ($P < 0.05$) and the

coefficient that relates the covariate and the pharmacokinetic parameter was significantly different than 0 ($P < 0.05$).

Based on the individual estimated pharmacokinetic parameters obtained from the final model (including covariates), we obtained the simulated plasma concentration versus time data for topotecan and melphalan to calculate by means of the log-linear trapezoidal rule the systemic exposure (area under the concentration versus time profile) for each cycle that a pharmacokinetic study was performed.

In a previous study, we characterized melphalan pharmacokinetics after unilateral and bilateral SSOAI administrations as described elsewhere.[6] Until the new schema of combined melphalan and topotecan chemotherapy was implemented, patients received melphalan alone, at the same reported dosages,[6] and pharmacokinetic studies were carried out in consenting patients. Thus, our previous pharmacokinetic data consisted of plasma concentrations obtained in 39 cycles from 19 consenting patients after melphalan single-agent SSOAI. These data were used as a control group to assess the effect of concomitant topotecan on melphalan pharmacokinetics with statistical significance.

Statistical Analysis

Linear mixed effects models were used to determine the association between melphalan systemic exposure and dosage using R-project (R Project for Statistical Computing; available at: http://www.r-project.org/; accessed June 4, 2013).

For the safety analysis, we calculated the incidence of hematologic toxicity as the ratio between the number of cycles in which patients demonstrate grade III and IV neutropenia with respect to the total number of cycles analyzed in the study. In addition, we compared this proportion with our previous data published elsewhere[6] regarding patients demonstrating severe neutropenia after receiving less than 0.5 mg/kg of melphalan as a single agent, by means of the Fisher exact test at a significance level of $P < 0.05$. In addition, we calculated the sample size needed to provide statistical significance when comparing the incidence of grade III/IV neutropenia after melphalan single-agent treatment with respect to the association with topotecan. The significance level was set at 0.05 (type I error) with a power of 0.9 (type II error). We considered acceptable a rate of grade III/IV neutropenia lower than 50% (as previously obtained with single-agent melphalan at doses of more than 0.5 mg/kg). Thus, at least 34 cycles were needed to confirm whether the difference in treatments truly existed.[6,23] Finally, we also conducted a power analysis to calculate the required sample size for comparison of melphalan pharmacokinetic parameter means obtained after single-agent infusion and concomitant with topotecan. We took into account the significance level of 0.05 (type I error) and a power of 0.8 (type II error); the difference of means was assumed to be significant if 25% in clearance could be detected when adding topotecan to the infusion of melphalan. Finally, equal standard deviation of melphalan clearance in both groups was assumed for the analysis. Kaplan-Meier ocular survival analysis was performed using GraphPad Prism software (GraphPad, San Diego, CA).

Results

Patient Population

A total of 26 patients (27 eyes) received 66 SSOAI sessions of melphalan and topotecan in combination. The median age at the time of the first cycle was 1.6 years (range, 0.8−7.4 years). The characteristics and demographics of the patients are summarized in Table 1. Overall, 5 eyes were treated with this combination as their primary treatment (n = 5 patients) or bridge therapy, and 22 eyes were treated with this combination because of relapsed or resistant disease. All cycles were evaluable for toxicity. Pharmacokinetic studies were carried out in 39 cycles on 21 patients. These studies were not possible in 4 consenting patients receiving only 1 cycle of chemotherapy because of the lack of a suitable vascular access and in 1 patient because the patient declined to consent to the procedure.

Table 1. Patient Demographics and Characteristics at the Start of Combination Chemotherapy

Feature	Median (Range) or No.
Age (yrs)	1.6 (0.8−7.4)
Weight (kg)	12 (8−30)
BSA (m^2)	0.51 (0.35−1.0)
Sex	
Male	12
Female	14
Previous treatment	
Total number of treated eyes	27
Systemic chemotherapy	22
SSOAI*	12
Bridge (group D)	2
First line (group D)	3
No. of cycles	66
Dose (mg)	
2	1
3	26
3.5	2
4	19
5	8
6	8
7	1
8	1

BSA = body surface area; *SSOAI = ophthalmic artery superselective infusion with topotecan and carboplatin or melphalan single-agent administered in previous cycles to melphalan plus topotecan combination therapy.

Clinical Response to the Topotecan and Melphalan Combination

Overall, of the 27 evaluated eyes, 21 (77.8%) responded to the melphalan plus topotecan combination. A representative eye that responded to the melphalan and topotecan association is shown in Figure 1. Response of eyes that were treated with primary or bridge therapy was better (5/5) than that of eyes with relapsed or resistant disease (16/22). All eyes treated with primary or bridge therapy and 13 of the 22 relapsed or resistant eyes were preserved at the time of this report, with a median follow-up of 11.7 months (range, 7.0−20.6 months). The probability of ocular survival at 1 year was 0.64 (95% confidence interval [CI], 0.4−0.8) for all eyes and 0.55 (95% CI, 0.28−0.75) for relapsed or resistant eyes (Fig 2).

Melphalan Pharmacokinetics

A total of 177 melphalan plasma concentrations from 21 patients were used to develop the pharmacokinetic model. Based on visual inspection of the concentration versus time data and statistical parameters, a 2-compartment model adequately fitted the melphalan plasma data. The pharmacokinetic parameters estimated included clearance (volume of plasma of drug cleared per unit of time), volume of distribution of the central and peripheral compartment (hypothetical volumes in which the drug is dissolved), and intercompartmental clearance assuming a log-normal distribution.

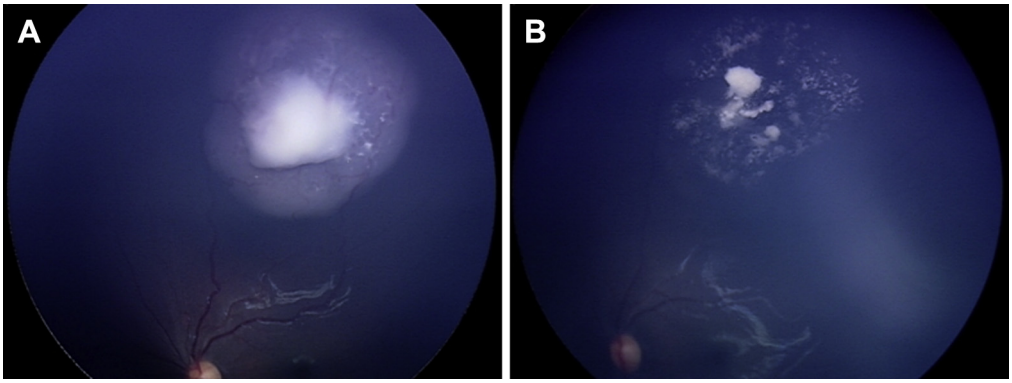

Figure 1. Fundus photographs of an eye from a child with unilateral retinoblastoma treated with melphalan plus topotecan superselective intra-arterial infusion as primary treatment showing tumor features (**A**) at diagnosis and (**B**) after 2 cycles of this combination chemotherapy. A marked reduction in tumor size and vascularization as well as therapy-induced calcification was observed after 2 cycles.

Subsequently, a covariate analysis was carried out to determine the influence of demographic and clinical parameters in melphalan pharmacokinetics. Specifically, normalizing the dose to body weight resulted in an improvement of the model and explained 49% and 66% of the interindividual variability in clearance and volume of distribution of melphalan, respectively. Thus, the dose subsequently was normalized to weight, as routinely performed in the clinics. Other demographic covariates did not improve the model further, and thus only weight was retained with statistical significance ($P < 0.001$). Melphalan normalized population parameters are reported in Table 2. Depicted in Figure 3A are the individual plasma concentration versus time data; the solid line represents the model prediction for a typical patient with a body weight of 10 kg who received 3 mg SSOAI of melphalan concomitant with topotecan.

Interestingly, a relationship between systemic exposure to melphalan (area under the concentration versus time profile) and dose corrected by weight was observed as depicted in Figure 4A and in correspondence to our previous reports of single-drug melphalan administration based on linear mixed-effects modeling ($P < 0.05$).

Topotecan Pharmacokinetics

We characterized topotecan pharmacokinetics after SSOAI using 173 plasma concentrations from 21 patients for developing the model. Population pharmacokinetics of topotecan was best described by a 2-compartment model (Fig 3B) and using an additive residual error model in concordance with the limit of quantitation. As previously described for melphalan, topotecan pharmacokinetic parameters were normalized by body weight because a statistically significant association was observed ($P < 0.001$). Mean population pharmacokinetic parameters (standard error) obtained for the final model are described in Table 2. Still, substantial interindividual (59%) and interoccasion variability (70%) in topotecan clearance remained unexplained, although no other demographic parameter could explain this variability statistically.

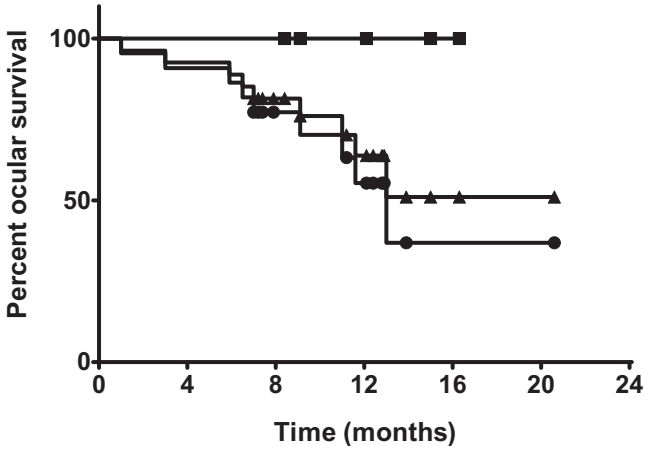

Figure 2. Kaplan-Meier ocular survival curves for (▲) all patients, (■) bridge therapy and chemotherapy-naïve patients, and (●) refractory/resistant eyes.

Effect of Sequence on Melphalan and Topotecan Pharmacokinetics

Patients who consented to pharmacokinetic studies were given indistinctly 1 of 2 sequences of treatment during each cycle of chemotherapy. In 20 and 19 cycles, patients were infused with melphalan and then with topotecan or with topotecan first and then melphalan, respectively. As shown in Figure 4B, no statistically significant difference was evident when comparing melphalan systemic exposure with respect to the sequence of drug administration ($P > 0.05$).

Effect of Topotecan on Melphalan Pharmacokinetics

In a previous study, we characterized melphalan pharmacokinetics after unilateral and bilateral infusion, and these data were used as the control group. Thereafter, we performed a population pharmacokinetic analysis using a nonlinear mixed-effects model, including melphalan plasma concentration versus time data from patients who received single-agent melphalan and the combination with topotecan. Then, topotecan was analyzed as a covariate to determine whether it had an effect on melphalan pharmacokinetics. As shown in Figure 4C, concomitant administration of topotecan had no effect on melphalan pharmacokinetics and specifically on the systemic exposure ($P > 0.05$).

Toxicity

A total of 66 cycles for 26 patients infused with melphalan and topotecan in combination were available from evaluation of toxicity. The median dose of melphalan was 0.30 mg/kg (range, 0.16−0.44 mg/kg), as presented in Table 3. In general, the combination therapy of melphalan and topotecan SSOAI was well tolerated and very few adverse events were recorded. There was no grade III or greater ocular toxicity. There were 7 episodes of grade III neutropenia in 5

Table 2. Melphalan and Topotecan Population Pharmacokinetic Parameters after Concomitant Superselective Ophthalmic Artery Infusion in Retinoblastoma Patients

Parameter	Melphalan Concomitant with Topotecan	Topotecan	Melphalan Single Agent*
Clearance (l/hour/kg)	0.44 (0.02)	0.67 (0.072)	0.51 (0.03)
V_c (l/kg)	0.23 (0.02)	0.53 (0.09)	0.18 (0.04)
Q (l/hour/kg)	0.67 (0.14)	2.86 (0.34)	0.93 (0.15)
V_p (l/kg)	0.26 (0.02)	0.72 (0.07)	0.25 (0.03)
AUC/D (ng*h/ml/mg†)	150.8 (68.6–321.4)	95.5 (34.5–237.9)	165.5 (83.8–397.6)

AUC/D = area under the concentration versus time profile corrected by dose (D); Q = intercompartmental clearance; V_c = volume of distribution of the central compartment; V_p = volume of distribution of the peripheral compartment.
Data are mean (standard error) unless otherwise indicated.
*Data previously published.
†Data are median (range).

patients and only 1 case of grade IV neutropenia, but no child had to be hospitalized for fever or neutropenia. Thus, taking into account all patients, the incidence of grade III and IV neutropenia was 10.6% and 1.5%, respectively. Specifically, 7 cycles after melphalan and topotecan SSOAI were evaluated in bridge therapy, whereas 10 cycles were evaluated in chemotherapy-naïve patients. None of the 7 cycles in bridge patients and only 1 of 10 cycles in chemotherapy-naïve patients resulted in grade III neutropenia. No patient required transfusion of blood products. Besides the hematologic toxicity, a grade III toxicity consisting of cranial nerve III palsy that resolved with corticosteroids occurred in 1 patient. Finally, no patient demonstrated metastatic disease, and all were alive at the time of this evaluation.

Interestingly, when comparing these results with our previous data obtained in 49 cycles from 21 patients who received SSOAI melphalan in doses of less than 0.5 mg/kg, the incidence of neutropenia was 16.3%. In that previous cohort, a total of 3 episodes of grade III and 5 of grade IV neutropenia were recorded. Hence, the incidence of neutropenia did not change when topotecan was administered concomitant with melphalan ($P > 0.05$).

Discussion

In this study, we found that the combination of melphalan and topotecan for SSOAI treatment of retinoblastoma was effective and well tolerated without increased hematologic toxicity with respect to melphalan administered as a single drug. Melphalan pharmacokinetics was not affected by the association with topotecan, and the sequence of drug administration did not influence melphalan systemic exposure. The systemic disposition of the proposed dose for topotecan administered by SSOAI is described.

The clinical implications of our findings include the description of an active chemotherapy combination regimen for the treatment of retinoblastoma using SSOAI. However, the ultimate efficacy of this approach compared with melphalan alone or other combinations in terms of ocular salvage were beyond the aims of this study. Besides, we acknowledge that statistical improvement in eye preservation rate by adding topotecan to melphalan SSOAI could be proved only by a randomized comparison, which has never been done for this pediatric population. In that sense, our speculations of a synergistic effect with topotecan were based on our previous work in retinoblastoma cell lines, and here we described the safety pattern of the drug association and the pharmacokinetic behavior of topotecan and melphalan.

Our study provides solid data on the pharmacokinetics of this combination by characterizing the systemic disposition of each infused drug that can be associated to systemic toxicity.

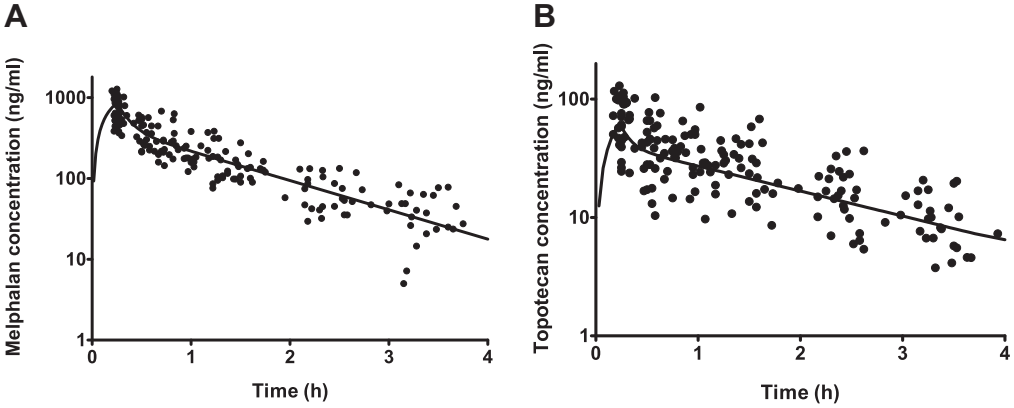

Figure 3. **A**, Graph showing melphalan concentration versus time profile after superselective ophthalmic artery infusion (dose, 0.32 mg/kg) in a unilateral retinoblastoma patient. The symbols represent melphalan plasma observed concentrations and the lines represent the model-predicted concentrations. **B**, Graph showing topotecan concentration versus time profile after superselective ophthalmic artery infusion (dose, 0.08 mg/kg) in a unilateral retinoblastoma patient. The symbols represent topotecan plasma observed concentrations and the lines represent the model-predicted concentrations. h = hours.

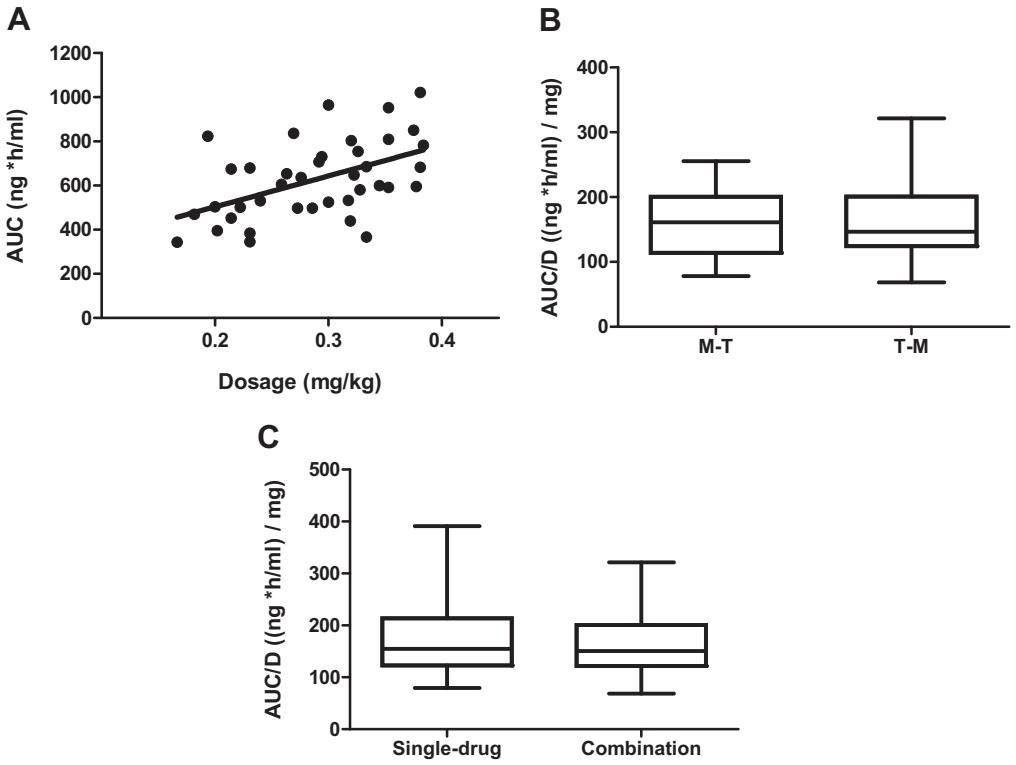

Figure 4. **A**, Graph showing the relationship between systemic exposure of melphalan (area under the concentration versus time profile) and dosage after superselective ophthalmic artery infusion (SSOAI). **B**, Graph showing comparison between melphalan systemic exposure corrected by dose (AUC/D) and the sequence of drug administration. **C**, Graph showing comparison melphalan systemic exposure corrected by dose (AUC/D) with and without topotecan concomitant administration. h = hours; M-T = melphalan and then topotecan SSOAI; T-M = topotecan and then melphalan SSOAI.

For the past 5 years, SSOAI has been under debate and has seen an exponential increase in its use for intraocular retinoblastoma.[1–9] However, relatively little is known about the pharmacokinetics of the drugs, their systemic toxicity, the schedule of treatment, and the alternative associations of drugs. This regimen was based on our previous experience showing a synergistic activity of this combination in retinoblastoma cell lines, along with a favorable ocular pharmacokinetic profile for topotecan based on our results in non-tumor-bearing animal models.[6] In those models, although topotecan showed a preferential passage to the vitreous, where it stayed for at least 4 hours over its calculated IC50 (50% inhibitory concentration)[13] melphalan showed a less favorable penetration into the vitreous but higher levels in the retinal pigment epithelium, which could explain its excellent efficacy for the treatment of eyes with retinal detachment caused by the tumor.[24] In addition, we previously reported the impact of melphalan SSOAI dose reduction on simulated vitreous concentrations attained in a hypothetical patient under tandem infusion based on the ocular disposition in an animal model. Specifically, when limiting the dose to 0.5 mg/kg with respect to the calculated dose based on weight to avoid severe neutropenia, the systemic exposure decreases, but also lower vitreous concentrations should be expected after the infusion.[6]

Table 3. Population Characteristics and Safety Parameters

Treatment/Parameter	Melphalan Concomitant with Topotecan	Single-Drug Melphalan
No. of patients	26	21
No. of cycles	66	49
Mean age (range), yrs	1.6 (0.8–7.4)	1.7 (0.5–6.2)
Mean weight (range), kg	12 (8–30)	10.1 (8.0–20.5)
Mean dosage (range), mg/kg	0.30 (0.16–0.44)	0.38 (0.24–0.50)
No. of cycles with severe neutropenia	8	8
Incidence of neutropenia (%)	12.1	16.3

Overall, to avoid increasing the melphalan dose without losing pharmacologically active exposure in the vitreous, we proposed the combination of melphalan and topotecan. These features favored the introduction of this regimen for clinical use by our group for the treatment of high-risk children with retinoblastoma. However, the toxicity profile of this combination and the potential pharmacologic interactions were not known. We knew from previous experience the systemic disposition of melphalan after SSOAI single-agent administration, but no information about topotecan pharmacokinetics after this relatively new route of infusion was available at the time of the present study. Plasma pharmacokinetic studies in this situation are useful to predict systemic toxicity, but because ocular pharmacokinetic studies cannot be performed in patients, no evaluation of the attained levels and disposition in the eye is possible, so we extrapolate the data from our animal

studies.[6,13] The data obtained in the present study after topotecan SSOAI are in agreement with total topotecan pharmacokinetic data previously reported in children. Mean systemic clearance of total topotecan was 0.67 l/hour per kilogram in our patients, or the equivalent of 8 l/hour if taking into account a mean population weight of 12 kg. This value is in concordance with a clearance of 9 to 10 l/hour previously reported in children infused with topotecan over 30 minutes for 5 days weekly and calculated based on an average body mass surface of 1.2 m^2.[25,26] Thus, topotecan pharmacokinetics is unchanged whether it is administered as an intravenous infusion or SSOAI in pediatric patients. Of note is that systemic exposure to topotecan lactone has been reported to be approximately 30% of total topotecan (lactone plus carboxylate).[10,25] Hence, because we reported herein a median systemic exposure of 95 ng*h/ml for total topotecan, we speculated about a topotecan lactone median systemic exposure of approximately 30 ng*h/ml after each cycle of SSOAI. This value is important because severe hematologic toxicity has been reported to occur when systemic exposure equals or exceeds 180 ng*h/ml, an amount that could hardly be expected in our patients after 1 mg of topotecan SSOAI.[26]

In addition, combining topotecan with melphalan forced us to reduce by half the length of infusion of each drug compared with our previous schedule. Decreasing the time for angiographic exposure and anesthesia is advisable for retinoblastoma patients based on their increased susceptibility to second tumors because of irradiation.[27,28] Thus, it has been suggested previously in different reports that chemotherapy infusion should last less than 30 minutes per eye treated.[4,9] Compared with our previous reports of systemic pharmacokinetics of melphalan after 30 minutes of single-drug infusion, we observed no difference in the pharmacokinetic parameters of melphalan between studies.[6] Although the rate of infusion does not seem to affect the systemic exposure, a clear correlation between dosage and this parameter was defined statistically; thus, we did not perform a prospective study comparing melphalan systemic pharmacokinetics in patients infused over 30 minutes with respect to a shorter interval of 15 minutes.

Of note is that potential pharmacokinetic interactions between melphalan and topotecan were unknown. Specifically, melphalan clearance is inversely related to the systemic exposure, and thus a change in the pharmacokinetic parameter resulting from drug−drug interaction could determine a different pattern of hematologic toxicity when the combination is used. That could be the case in which systemic exposure to melphalan increases with concomitant infusion of topotecan, and thus the myelosuppression expected for those cycles changes according to the pharmacokinetic parameter.[14] However, melphalan pharmacokinetics were not affected by the addition of topotecan because no change in the pharmacokinetic parameters was observed ($P > 0.05$). In correspondence with comparable systemic exposures, the incidence of severe neutropenia (grade III and IV; 12%) was comparable with that obtained in a previous cohort of 21 patients (49 cycles) who received only SSOAI melphalan as a single agent at doses of less than 0.5 mg/kg as a reference group ($P > 0.05$). The worst scenario would be an incidence of 50% of severe neutropenia, as we reported previously for melphalan single-agent SSOAI administered at doses of more than 0.5 mg/kg.[6] However, that was not the case, and no significant difference in the proportion of neutropenia could be detected when adding topotecan to the treatment. Taken together, the present results show that the combined melphalan and topotecan SSOAI resulted in the same pattern and incidence of side effects as those previously reported for melphalan single-agent treatment, while introducing a potentially active drug for the treatment of this aggressive tumor.

Our study also provided data about the observed relationship between melphalan systemic exposure with respect to dosage despite concomitant administration of topotecan. As previously reported for melphalan administered as a single agent for SSOAI, a linear increase in melphalan systemic exposure with respect to dosage was observed. The estimated population pharmacokinetic parameters obtained in the present study (Table 2) were in the same range as those reported by others after intravenous administration in children with malignant disease undergoing stem cell transplantation.[15] Thus, despite assuming that SSOAI is a local route for drug dosing to the eye, systemic melphalan pharmacokinetics do not differ between SSOAI and the intravenous administration in different pediatric populations.

We also investigated if there was any sequence effect in the plasma pharmacokinetics of this combination. Melphalan SSOAI causes significant alterations in the eye vasculature during infusion.[19] Thus, it is possible that the pharmacokinetics of other drugs administered after melphalan may be limited by this fact. The issue of the sequence of drug administration is important because melphalan should be reconstituted immediately before infusion because of its instability.[16] If melphalan is to be administered first, the entire procedure may take longer to account for the time of drug reconstitution. Topotecan may be reconstituted in advance because its solubility and stability allow for preparation without losing efficacy.[16] Because we found no sequence effect on plasma pharmacokinetics, topotecan may be infused first while melphalan is reconstituted by another operator or in the pharmacy on immediate call.

In conclusion, a regimen combining melphalan and topotecan for SSOAI treatment of retinoblastoma is active and well tolerated. This combination therapy theoretically allows potentiation of the pharmacologic activity of both drugs and enhanced tumor control, while not increasing hematologic toxicity. We did not evidence a pharmacokinetic interaction between melphalan and topotecan, and the sequence of drug administration did not influence melphalan pharmacokinetics. Topotecan systemic exposure after SSOAI of the current dose is not expected to yield hematologic toxicity. Additional studies are needed to compare its efficacy with that of other regimens.

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