Combined Intravitreal Melphalan and Topotecan for Refractory or Recurrent Vitreous Seeding From Retinoblastoma

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Importance
Demonstrating the usefulness and complications of multiagent intravitreal chemotherapy is necessary for successful treatment in patients with recalcitrant vitreous seeding of retinoblastoma.

Objective
To determine the efficacy and complications of combined intravitreal chemotherapy (melphalan hydrochloride and topotecan hydrochloride) for viable vitreous seeding from retinoblastoma.

Design, Setting, and Participants
This retrospective study was conducted in a hospital setting. Trans-pars plana intravitreal injection of melphalan hydrochloride (40 μg in 0.04 mL of diluent) combined with topotecan hydrochloride (8-20 μg in 0.04 mL of balanced salt solution) was performed in 9 eyes, followed by injection site cryotherapy.

Main Outcomes and Measures
Complete regression of vitreous seeds of retinoblastoma.

Results
Nine eyes, initially classified as group D (n = 6) or E (n = 3) according to International Classification of Retinoblastoma categorization, received a standard 6 cycles of intravenous chemotherapy and/or intra-arterial chemotherapy and subsequently developed recurrent viable vitreous seeds. Intravitreal administration of melphalan combined with topotecan produced complete control of vitreous seeds in all 9 eyes following a mean of 1.9 injections (median, 2; range, 1-3 injections). In 3 cases (33%), tumor control was achieved with a single injection, whereas in 6 (67%) cases, 2 or 3 injections were necessary. Three patients (33%) subsequently underwent enucleation because of recurrent tumor and persistent anterior chamber lesions. During a mean 15.2 months of follow-up (median, 16; range, 7-25 months), there was no recurrence of new tumor or vitreous seeds in the remaining 6 eyes. Complications included temporary hypotonia of 2 weeks or less (2 [22%]), temporary epithelial defect (1 [11%]), and vitreous hemorrhage (1 [11%]). There was no case of episcleral or orbital retinoblastoma extension or remote retinoblastoma metastasis. There was no change in the a and b waves of bright-flash electroretinograms.

Conclusions and Relevance
Administration of combined intravitreal melphalan and topotecan in eyes not subsequently enucleated appears to be safe and effective for resistant or recurrent vitreous seeds from retinoblastoma. In 3 of the cases (33%), tumor control was achieved with a single injection.
In the current era of retinoblastoma management, there are several alternatives for treatment of viable vitreous or subretinal tumor seeds, including intravenous, intra-arterial, subtenon, or intravitreal chemotherapy, as well as enucleation or external beam radiotherapy. The choice of therapy is especially critical if the eye of concern is the only remaining eye of a young infant. All eyes with extensive vitreous seeds are classified as group V by the International Classification of Retinoblastoma or group D or E by the International Classification of Retinoblastoma. Previous reports investigating intravenous chemotherapy for retinoblastoma have delineated success (globe salvage and avoidance of external beam radiotherapy) in 83% to 87% of groups I through IV and 36% to 47% of group V eyes. Success with intravenous chemotherapy, based on the International Classification of Retinoblastoma, was 90% to 100% for groups A, B, and C; 47% for group D; and 25% for group E. These results underscore the difficulty in vitreous seed control using intravenous chemotherapy.

Results for retinoblastoma control by intra-arterial chemotherapy have been somewhat more favorable for eyes with advanced disease, with globe salvage in 100% of group D and 33% of group E eyes. Shields and associates documented that intra-arterial chemotherapy can lead to complete control in 82% of subretinal seeds and 67% of vitreous seeds. In a similar study by Abramson and associates, among treatment-naive eyes, the 2-year probability of globe salvage in eyes with retinoblastoma and subretinal seeding was 83%; in vitreous seeding, 64%; and in eyes with both subretinal and vitreous seeding, 80%. The above studies indicate that retinoblastoma and vitreous seeds remain uncontrolled following intravenous chemotherapy in approximately 33% to 36% of eyes.

Direct injection of chemotherapy agents into the vitreous cavity (intravitreal chemotherapy [IVC]) for vitreous seed control has gained substantial attention in the literature. Melphalan hydrochloride is the most commonly used medication, but others, including thiotepa, methotrexate, and most recently, topotecan hydrochloride, have been administered.

Previous work identified the risks and benefits of melphalan for vitreous seed control and explored complications based on medication dose. A dose of 8 μg was relatively ineffective and 50 μg was effective but toxic, leading to hypotony and phthisis bulbi. A dose of 20 to 40 μg was the ideal balance of efficacy with minimal toxic effects.

Topotecan is a topoisomerase I inhibitor that can produce lethal damage in DNA replication of cells and has shown activity in pediatric tumors, such as retinoblastoma, when administered via the intravenous and intra-arterial routes. We explored the efficacy and complications of intravitreal topotecan in combination with melphalan for control of recurrent or refractory vitreous seeds from retinoblastoma.

Methods

This retrospective study was approved by the Farabi Hospital Review Committee of Tehran University of Medical Sciences, Tehran, Iran. Criteria for inclusion were eyes with retinoblastoma that showed persistence or recurrence of viable vitreous seeds following standard systemic chemotherapy. In all cases, enucleation was the remaining option. After written informed consent for participation in the study and oral informed consent for use of IVC, all patients received IVC with combined melphalan and topotecan. Intravitreal melphalan hydrochloride (Alkeran), 40 μg, in 0.04 mL of diluent and topotecan hydrochloride (Hycamtin), 8 to 20 μg, in 0.02 in 0.04 mL of balanced salt solution was injected under sterile technique. In 4 patients, combined IVC was used as the first step of IVC (Table 1). In the other 5 patients, who had recalcitrant vitreous seeds after systemic chemotherapy and multiple previous intravitreal melphalan, combined chemotherapy (melphalan and topotecan) was administered. Both drugs were prepared in the operating room, where the patient was being examined while under general anesthesia. The drugs were reconstituted in a sterile fashion on a separate sterile tray by combination of the freeze-dried chemotherapy powder with diluent.
ent provided by the manufacturers for melphalan and powder with balanced salt solution for topotecan. The initial dose of topotecan hydrochloride was 8 μg in the first patients; later dose escalation for improved vitreous seed control led to an adjustment up to 20 μg (Table 2). After preparation, the 2 medications were separately injected promptly through the pars plana (2.5 mm from the limbus, with a beveled approach) superotemporally using a 30-gauge needle. There was no aqueous humor tap to soften the globe. Cryotherapy at the injection site was performed during needle withdrawal as a single freeze-thaw cycle. Globe “jiggling” to disperse chemotherapy within the eye was performed for 10 seconds. An antibiotic and corticosteroid preparation was applied to the eye after the injection. There was no eye patch or medication prescribed postoperatively. To prevent infection, the family was instructed to not manipulate the eye.9

Intraocular pressure was monitored 30 minutes after injection and at each follow-up. Patient examination was performed every 2 weeks under general anesthesia, with repeated injection if needed at the time of general anesthesia until complete vitreous seed control was achieved. Success was defined as complete regression of vitreous seeds clinically. Failure, independent of eyes undergoing enucleation, was defined as vitreous seed nonresponse, increase in the amount of vitreous seeds, and/or the appearance of new vitreous seeds noted on ophthalmoscopic examination.

Results

During the past 2 years, 9 eyes of 9 patients received combination IVC with melphalan and topotecan for viable vitreous seeds from retinoblastoma (Table 2). All 9 patients had received 6 cycles of standard chemotherapy (vincristine sulfate, etoposide, and carboplatin). In addition, 1 patient received external beam radiotherapy and 5 received intra-arterial chemotherapy for tumor and seed control. Four of the 9 patients had previously undergone enucleation of the opposite eye for advanced retinoblastoma. At the time of intravitreal injection, no patient was receiving systemic chemotherapy.

The intravitreal injection was uncomplicated in every case. A total of 40 injections were given through the pars plana. There were no immediate or delayed complications of vitreous hemorrhage, retinal detachment, endophthalmitis, or cataract. At 30 minutes following the injection and at the 2-week follow-up, intraocular pressure was 22 mm Hg or less. There were 2 cases of hypotony (intraocular pressure decreased to 8 mm Hg), lasting for 2 weeks. One of those eyes was later enucleated.

Success with complete control of vitreous seeds was achieved in all 9 cases (100%) with a mean of 1.9 injections of topotecan and melphalan (median, 2; range, 1-3 injections) (Figure). In 3 cases (33%), tumor control was achieved with a single injection, whereas in 6 (67%) cases, 2 or 3 injections were necessary. In case 9, an 8-μg dose of topotecan hydrochloride was used for 2 sessions, but it was not effective, and the dosage was increased to 20 μg. Five of the 9 eyes (56%) had received previous intravitreal monotherapy with melphalan for a mean of 4.6 injections (median, 5; range, 2-8 injections) without significant response to treatment before the decision of combined IVC was made.

In 3 patients (33%) enucleation was performed: 1 with anterior segment retinoblastoma and the other 2 with recurrent intraretinal tumor near previously regressed retinoblastoma after both intravenous and intra-arterial chemotherapy. The pathology reports showed the active tumor on the iris and ciliary body in one patient, with adjacent vitreous involvement.

### Table 2. Successful Combined IVC Melphalan Hydrochloride and Topotecan Hydrochloride for Retinoblastoma a

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Time Between IV or IAC and IVC, mo</th>
<th>No. of IVC Sessions</th>
<th>Dose, μg</th>
<th>Additional Treatment (Reason)</th>
<th>Follow-up After Completion of IVC, mo</th>
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<td></td>
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<td>Melphalan Before Combined Therapy</td>
<td>Melphalan With Topotecan</td>
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<td>9</td>
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<td>3</td>
<td>40</td>
<td>8 (2 sessions); 20 (2 sessions)</td>
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</tbody>
</table>

Abbreviations: EBRT, external beam radiotherapy; IAC, intra-arterial chemotherapy; IV, intravenous; IVC, intravitreal chemotherapy; NA, not applicable.

* Success was considered a decreased amount or disappearance of vitreous seeds.

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and the active tumor in the retina with a small amount of vitreous seeds near the new lesion.

Complications with doses of topotecan hydrochloride, 8 to 20 μg, and melphalan hydrochloride, 40 μg, were mild, including temporary hypotonia in 2 patients for 2 weeks, epithelial defect in 1 patient lasting 1 week, and a mild vitreous hemorrhage in 1 patient. There were no a- and b-wave changes in the bright-flash electroretinogram in our patients. In addition, there was no subconjunctival, orbital, or metastatic retinoblastoma during a mean of 15.2 months follow-up (median, 16; range, 7–25 months).

Discussion

Viable retinoblastoma seeding into the vitreous after intravenous or intra-arterial chemotherapy has been a major obstacle to globe salvage. Intravitreal chemotherapy was first explored by Ericson and Rosengren in 1961. Intravitreal injection of melphalan hydrochloride, 8 to 30 μg, combined with ocular hyperthermia was promoted by Suzuki and Kaneko, who showed promising results with nearly 51% success. Munier and associates explored intravitreal melphalan hydrochloride, 8 to 30 μg, in 23 patients with recurrent retinoblastoma vitreous seeds, using up to 8 weekly injections, and achieved 87% control at 22 months.

Ghassemi and Shields studied 12 children facing enucleation for viable persistent or recurrent vitreous seeds following systemic chemotherapy. Eight eyes treated with 8 to 10 μg of melphalan hydrochloride showed partial control at 71% short-term and 43% long-term follow-up, with minor local toxic effects. In that study, 4 eyes received 50-μg doses, with 100% control in short- and long-term follow-up, but with serious adverse local effects of hypotonia and/or phthisis bulbi in some cases. More recently, Shields and associates reported 100% control of retinoblastoma vitreous seeds in 11 additional cases, with minimal toxic effects, using a mean of 5 injections of melphalan hydrochloride, 20 to 30 μg. In the present study, retinoblastoma vitreous seed control was achieved in 100% of 9 eyes, using 40 μg of melphalan hydrochloride and 8 to 20 μg of topotecan hydrochloride, but with a much shorter course (median, 1 injection). The rapidity and duration of control with the single injection of 2 medications, with minimal toxic effects, were remarkable.

Topotecan displays excellent antitumor activity for retinoblastoma and relatively low ocular toxic effects. In a study of the pharmacokinetics of topotecan in non–tumor-bearing rabbit eyes, Buitrago and associates found that 4 weekly injections of 2 different doses of intravitreal topotecan hydrochloride (5 μg/0.1 mL and 0.5 μg/0.1 mL of diluent) into the eye of each rabbit showed no functional or histopathologic change in the retina and no systemic toxic effects. Topotecan has been demonstrated to have remarkably rapid and potent activity against human retinoblastoma in vivo and in vitro. Buitrago and associates found that intravitreal injection of 0.5 mg/0.1 mL and 5 mg/0.1 mL of diluent of topotecan hydrochloride resulted in substantial improvement of topotecan bioavailability in the vitreous of the rabbit eye compared with the local periocular route or intravenous administration, with the additional advantage of a 200-fold reduction in the administered dose. After injection of 0.5 mg/0.1 mL, maximum concentrations in the vitreous were 709.0 ng/mL and 695.4 ng/mL, as lactone and total topotecan, respectively. Vitreous levels above a potentially therapeutic threshold of 14.0 ng/mL were obtained for lactone topotecan up to 5.5 hours after intravitreal injection. This pharmacokinetic study showed that topotecan elimination half-life in the vitreous was 2.5 hours after a 5-mg dose; thus, if the drug is administered in a weekly fashion no accumulation should be expected.
Buitrago and associates found that this dose led to potentially therapeutic levels that significantly exceed the topotecan 50% inhibitory concentration for retinoblastoma up to 16 hours after administration. Moreover, low levels of topotecan in the plasma suggested that there might be minimal systemic-related adverse events. In the present study, a total of 17 combined sessions of intravitreal injections using combined melphalan and topotecan were given for complete response of vitreous seeds in 100% of eyes with minimal toxic effects. Previous protocols called for 6 or 8 injections of melphalan. In this series, we achieved vitreous seed control with a median of 2 injections (mean, 1.9), which is notably fewer than in previous studies. An additional finding in this study was that eyes with previous failure following intravitreal administration of melphalan showed a complete response to injection of the melphalan and topotecan combination.

Conclusions

Our 2-year experience with combined intravitreal injection of melphalan and topotecan for recurrent or recalcitrant vitreous retinoblastoma seeds has demonstrated seed control among eyes not subsequently enucleated, although the long-term visual outcome remains to be determined. This approach should be considered in children with retinoblastoma and vitreous seeding who do not respond to standard measures of intravenous or intra-arterial chemotherapy.

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Author Contributions: Dr Ghassemi had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Ghassemi, Roohipoor.
Acquisition, analysis, or interpretation of data: Ghassemi, Shields, Ghadimi, Khodabandeh.
Drafting of the manuscript: All authors.
Critical revision of the manuscript for important intellectual content: Ghassemi, Ghadimi.
Statistical analysis: Ghassemi, Shields, Ghadimi.
Obtained funding: Ghassemi.
Administrative, technical, or material support: Ghassemi, Shields, Khodabandeh, Roohipoor.
Study supervision: Ghassemi.
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REFERENCES

**OPHTHALMIC IMAGES**

**Infliximab Infusion Resulting in Rapid Resolution of Episcleritis**

Shokufeh Tavassoli, BSc(Hons), MBChB(Hons); Guy T. Smith, BSc(Hons), MBBS(Hons), FRCPhtth

**A**. Right-sided recurring episcleritis unresponsive to topical nonsteroidal anti-inflammatories (ketorolac tromethamine 3 times daily for previous 2 months) or topical steroids (flurometholone 4 times daily for previous month) in a 44-year-old female patient with Crohn disease, psoriasis, and enteropathic arthropathy. **B**. Marked reduction in redness within 5 hours of completion of an infliximab infusion. Within 24 hours, there was full symptomatic and clinical resolution, with no further recurrence.