Graefe's Archive for Clinical and Experimental Ophthalmology (2023) 261:1167–1175
https://doi.org/10.1007/s00417-022-05903-3

ONCOLOGY

Addition of intravitreal carboplatin with melphalan for management of vitreous seeding in retinoblastoma

Hamid Riazi-Esfahani[1,2] · Babak Masoomian[1] · Alireza Khodabandeh[1,2] · Abdulrahim Amini[2,3] · Sara Taghizadeh[1] · Leila Boujabadi[1] · Shervin Sharifkashani[1] · Carol L. Shields[4] · Fariba Ghassemi[1,2]

Received: 9 June 2022 / Revised: 25 October 2022 / Accepted: 4 November 2022 / Published online: 19 November 2022

Abstract

Purpose To evaluate the efficacy and toxicity of intravitreal carboplatin plus melphalan for the treatment of vitreous seeds in eyes with retinoblastoma (RB).

Methods This retrospective series at a tertiary referral center included 22 consecutive RB patients who had received intravitreal carboplatin (16 µg per 0.05 ml) combined with melphalan (30 µg in 0.03 ml) [IVi (Ca-Me)] for treatment of vitreous seeds. Tumor control and drug toxicities were recorded.

Results There were 22 eyes of 22 patients, divided into primary group ($n=13$) without history of previous intravitreal chemotherapy (IViC) and refractory group ($n=9$) with history of previous IViC using melphalan and/or topotecan. The demographics and clinical findings of the primary and refractory groups did not differ significantly. The 6-month follow-up revealed complete vitreous seed control (77% vs. 89%, $p=0.47$). Vitreous seed recurrence was detected in 1 eye of each group at 6 months. During the next 18-month follow-up period, no recurrence of seed was observed. The response to IVi (Ca-Me) was not significantly influenced by previous IViC ($p=0.70$), primary systemic or intra-arterial chemotherapy ($p=0.45$), or the type of regression ($p=0.35$). The most common tumor treatment complications were retinal detachment (RD) ($n=2$), early hypotony ($n=2$) and late hypotony ($n=4$, unrelated), cataract ($n=2$), and severe pigment dispersion ($n=1$). Enucleation was performed in 8 eyes, for total RD ($n=1$), phthisis bulbi ($n=5$), and extensive solid tumor recurrence ($n=2$). There was no case of orbital invasion, systemic metastasis, or death.

Conclusion Based on this interventional case series for primary and refractory vitreous RB seeds, carboplatin plus melphalan therapy may be effective with few toxic side effects.

Keywords Retinoblastoma · Vitreous seeds · Intravitreal chemotherapy · Carboplatin · Melphalan · Topotecan

Key messages

- There are still certain cases with resistant retinoblastoma vitreous seeds even after chemotherapy.
- Intravitreal chemotherapy with carboplatin plus melphalan is an appropriate option for the treatment of primary and refractory vitreous seeds with few potential complications.
- We can consider carboplatin plus melphalan for intravitreal chemotherapy especially when access to other regimens is limited.

✉ Fariba Ghassemi
fariba.ghassemi@gmail.com

[1] Ocular Oncology Department, Farabi Eye Hospital, Tehran University of Medical Sciences, South Kargar Street, Qazvin Square, Tehran, Iran

[2] Retina Service, Farabi Eye Hospital, Tehran University of Medical Sciences, Tehran, Iran

[3] Department of Ophthalmology, School of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

[4] Ocular Oncology Service, Wills Eye Hospital, Thomas Jefferson University, Philadelphia, PA, USA

Introduction

In recent years, the approach to treating retinoblastoma (RB) has gradually evolved from preserving lives to preserving eyes and vision after intravenous (IVC), intra-arterial (IAC), and intravitreous chemotherapy (IViC) were introduced [1]. However, complete and uncomplicated management of subretinal or vitreous seeds is still debatable [2]. Compared to solid tumors and subretinal seeds, vitreous seeds respond less profoundly to intravenous chemotherapy, perhaps because they are located in the vitreous cavity, where the lack of vascularity leads to minimal drug access [3]. Studies have shown that vitreous seeds can show persistence or recurrence in approximately one-third of eyes even with satisfactory tumor control by IAC and IVC [2, 4, 5].

IViC has emerged in recent years as an effective treatment modality for recurrent or refractory vitreous seeding by delivering a higher concentration of drug in the vitreous cavity while avoiding systemic drug toxicity [6]. Melphalan hydrochloride is the most frequently used intravitreal drug for the treatment of vitreous seeds in patients with RB [7]. Munier et al. observed vitreous seed regression in 87% of eyes treated with intravitreal injection of 20–30 µg/0.1 ml melphalan [8]. Ghassemi and Shields proposed 20–30 µg/0.05 ml injection of melphalan for 6 consecutive biweekly injections, which demonstrated therapeutic efficacy with complete vitreous seed regression in all enrolled eyes and with limited toxicity [6]. Shortly afterward, intravitreal topotecan hydrochloride was introduced as another treatment option [9, 10]. Three-weekly intravitreal topotecan alone appeared safe and effective for the treatment of nonresponsive or recurrent vitreous seeds with no obvious ocular or systemic complications [10]. Ghassemi et al.'s study showed that a combination of 40 µg of melphalan and 8 to 20 µg of topotecan resulted in 100% RB vitreous seed control in 9 eyes with few injections (2 sessions) [1]. They concluded that simultaneous use of two drugs may achieve faster control with fewer adverse effects [1].

Carboplatin is a chemotherapeutic agent that has long been used intravenously, periocularly, and intra-arterially to treat RB [11–13]. In experimental transgenic mouse models, repeated intravitreal injections of 1 µg carboplatin exhibited little functional or pathologic side effects and excellent retinal tumor control [14, 15]. Based on in vitro models, compared to melphalan and topotecan, carboplatin has less cytotoxicity to the retinal pigmentary epithelium (RPE) cells [16]. There are few reports about the utilization of intravitreal carboplatin in human models. Karkhaneh et al. investigated the safety and efficacy of carboplatin (10 µm/0.05 ml intravitreal + 20 mg/1 ml subtenon) for vitreous seeds in a single case and found tumor regression after three injections [12]. Smith et al. evaluated the efficacy of combined intravitreal (5 µg/0.05 ml) and subtenon (30 mg/1 ml) carboplatin in 2 patients with refractory vitreous seeds [13]. They finally enucleated both eyes 1 month after the injection due to persistent vitreous seeds and an active primary tumor. Histopathology revealed no apparent toxicity in the anterior segment, retina, or optic nerve and no viable vitreous seeds [13].

In spite of few previous observations, carboplatin has not been used as a standard treatment for IViC in cases of non-responsive or recurrent RB vitreous seeds. Herein, we explore the use of intravitreal carboplatin with melphalan for primary or refractory vitreous seeds in eyes with RB. We describe efficacy and safety in 22 consecutive patients.

Materials and methods

This retrospective, interventional, noncomparative study on 22 eyes of 22 patients with RB evaluated the outcome of intravitreal injections of carboplatin plus melphalan [IVi (Ca-Me)] between July 2019 and March 2020, at Farabi Eye Hospital, Tehran University of Medical Sciences. Informed consent was obtained from the patients' parents or legal guardians. Institutional Review Board approval was obtained from Farabi Eye Hospital (IR.TUMS.FARABIH.REC.1397.030), and this study adhered to the tenets of the Declaration of Helsinki.

Collected data included the patient's age, sex, laterality, and tumor stage based on the International Classification of RB (ICRB groups A–E). Type (dust, sphere, cloud, or mix) and location of seeding, primary chemotherapy (IVC, IAC, or IViC), or any other additional treatments (e.g., laser thermotherapy or cryotherapy) and total number and dose of IVi (Ca-Me) injections also were recorded. The outcome measures were defined as clinical response of the seeds by changing their distribution pattern and any potentially related complications.

There were two groups including the primary group ($n = 13$ eyes) that had never received IViC and the refractory group ($n = 9$ eyes) that had previously undergone IViC with melphalan and/or topotecan. All patients with germline RB received IVC at standard doses of vincristine (0.05 mg/kg body weight on day 1), carboplatin (18.6 mg/kg body weight on day 1), and etoposide (5 mg/kg body weight on days 1 and 2) given every 4 weeks, for a total of eight cycles. Two or three cycles of IAC using melphalan (5 mg), topotecan (0.6–1 mg), and carboplatin (25 mg) were done for individuals with somatic RB who were older than 4 months of age. Transpupillary thermotherapy (TTT) or cryotherapy for focal consolidation of the residual retinal tumors was performed when necessary. All study patients underwent a complete eye examination before

administration of IVi (Ca-Me). Examination under anesthesia was performed bimonthly during the course of treatment and included anterior segment evaluation, fundus evaluation with indirect ophthalmoscopy, B-scan ultrasonography, and RetCam (Clarity, Pleasanton, CA) fundus photography. After tumor control was achieved, the interval between examinations under anesthesia was extended.

All enrolled patients in this study received IViC using carboplatin (16 µg, in 0.05 ml in a balanced salt solution) plus melphalan (30 µg in 0.03 ml of diluent), prepared in the operating room while the patient was under general anesthesia. The medicines were reconstituted in a sterile manner on a separate sterile tray. The injection site was carefully chosen to ensure there was no nearby tumor mass, vitreous seeds, or subretinal fluid within 3 clock hours of the injection site. After preparation, the 2 drugs were separately injected through the pars plana (2–3 mm from the limbus, with a beveled or two-step approach) mostly supero-temporally and infero-temporally using a 30-gauge (8 mm-length) needle. The needle pointing towards the center of the vitreous cavity and away from the anatomical location of the lens.

After each injection, globe "jiggling" was performed for 10 s to dispense drugs within the eye. In the case of subconjunctival vitreous prolapse, 10–20 µg/0.02 ml subconjunctival melphalan was injected.

After the injection, the eye was patched for 3 h, and a topical antibiotic, corticosteroid, and homatropine 2% eye drops were administered for 5–7 days. All patients were examined for any possible intraocular inflammation or infection the day after injection. Patients were re-examined under general anesthesia every 2 weeks, and intravitreal injections were administered until full vitreous seed control was obtained qualitatively.

Complete regression was defined as the complete disappearance or calcification of all vitreous seeds by at least 6 months of follow-up. Complications/toxicities after IVi (Ca-Me) injections were recorded.

Statistical analysis

Statistical analysis was performed with linear regression analysis, 2-tailed Student *t*-test, and the Pearson test (or the Spearman test for nonparametric variables). The statistical analysis was performed using SPSS version 25.0 software (IBM, Armonk, NY, USA). All *p* values less than 0.05 were considered statistically significant.

Results

There were 22 eyes of 22 patients with RB vitreous seeds, treated with 50 IVi (Ca-Me) injections, from July 2019 to March 2020. These were divided into the primary IViC group ($n=13$ eyes) and the refractory group ($n=9$ eyes). Nine eyes of 9 patients with bilateral RB and 13 eyes of 13 patients with unilateral RB were included in the study. The mean age at the time of presentation was 32 months (median, 25; range, 6–72 months). The patients had been treated with IVC ($n=3$), IAC ($n=6$), or both treatments ($n=13$). At the time of IViC (Ca-Me), there were no patients on concurrent IVC or IAC. Cryotherapy and transpupillary thermotherapy (TTT) for solid tumors were performed as adjuvant therapies for 17 eyes.

Intravitreal chemotherapy using carboplatin and melphalan was primary first-line therapy ($n=13$ eyes) for vitreous seeds and secondary therapy for refractory vitreous seeds ($n=9$ eyes). Of those 9 eyes with refractory vitreous seeds, there was a history of previous IViC including melphalan alone (1 eye) or melphalan plus topotecan (8 eyes). The patient's demographic and clinical characteristics are listed in Tables 1 and 2. Figure 1 shows three patients with refractory vitreous seeds who have been treated successfully with intravitreal injections of carboplatin plus melphalan.

A comparison (primary vs. refractory groups) revealed no difference regarding patients mean age (33 vs. 26 months, $p=0.70$), unilateral cases ($n=7$ vs. $n=5$ eyes, $p=0.83$), vitreous seed type (non-dust) ($n=7$ vs. $n=5$, $p=0.78$), mean number of affected quadrants ($n=3$ vs. $n=2.4$, $p=0.26$), and mean number of injections ($n=2.2$ vs. $n=2.3$, $p=0.92$). Based on the ICRB tumor grouping, there were 7 eyes ($n=4$ vs. $n=3$) in group C, 11 eyes ($n=5$ vs. $n=6$) in group D, and 4 eyes ($n=4$ vs. $n=0$) in group E. According to number of quadrants containing active vitreous seeds, it was detected in 4 quadrants ($n=6$ vs. $n=1$), 3 quadrants ($n=2$ vs. $n=1$), 2 quadrants ($n=4$ vs. $n=5$), and 1 quadrant ($n=1$ vs. $n=1$) involvement. A comparison of complete vitreous seed control between groups revealed no difference between them ($n=10/13$ vs. $n=8/9$, $p=0.47$) over the short-term (6 months) follow-up. From 13 eyes in the primary group, two eyes revealed partial response; therefore, standard intravitreal injections with melphalan and topotecan were started. Additionally, one of the eyes failed to respond and was enucleated 1 month after the last injection due to advanced disease with no vision potential. In the refractory group, only one eye out of nine displayed a partial response. This eye was enucleated due to significant hypotony and cataract. Despite complete response, recurrence of vitreous seeds was observed in 1 eye in each group, 4 months after the last IVi (Ca-Me) ($p=0.80$); therefore, intravitreal injections of melphalan and topotecan were initiated in both eyes (Tables 1 and 2).

Complete vitreous seed regression did not correlate with patient age ($p=0.75$) or baseline tumor features including basal diameter ($p=0.56$), thickness ($p=0.68$), or distance to the optic nerve ($p=0.54$). The response to IVi (Ca-Me) was not influenced by previous IViC ($p=0.70$). Complete

Table 1 Patients' demographics, clinical features, treatments, and outcomes in group with primary vitreous seeds treated with intravitreal carboplatin plus melphalan

Case no	Age, (month)	Laterality	Affected eye ICRB group	Other eye ICRB group	Type of vitreous seeds	Number of (Ca + Me) injections	No. of affected quadrants	Subretinal seed	Treatment response	Complications (6 months f/up)	Enucleation (6 months f/up)	Treatments used between 6 and 24 months of f/up	Complications (24 months f/up)	Enucleation (24 months f/up)
1	40	Unilateral	OD/E†	NA	Sphere	3	4	Yes	Failure	RRD	Yes	Nil	Nil	Nil
2	18	Bilateral	OD/C	D	Dust	2	3	No	Complete	None	No	None	None	No
3	39	Bilateral	OD/D	B	Dust	5	4	No	Partial	None	No	(M + T) × 2	Hypotonia	Yes
4	38	Unilateral	OS/E†	NA	Mixed	3	4	No	Partial	None	No	(M + T) × 1	Cataract	No
5	60	Unilateral	OD/D	NA	Mixed	1	4	No	Complete	None	No	Cryotherapy + TTT	None	No
6	16	Bilateral	OS/C	C	Mixed	2	2	No	Complete	Hypotonia	Yes	Nil	Nil	Nil
7	24	Unilateral	OS/E†	NA	Dust	2	2	No	Complete	None	No	None	None	No
8	14	Bilateral	OD/D	B	Dust	1	4	No	Complete	None	No	IVC + TTT + cryotherapy	None	No
9	19	Bilateral	OD/E†	NA	Mixed	1	2	Yes	Complete	None	No	IAC	Main tumor recurrence	Yes
10	15	Unilateral	OS/C	NA	Dust	3	1	Yes	Complete	None	No	None	None	No
11	25	Unilateral	OD/D	NA	Dust	1	3	Yes	Complete	None	No	None	Hypotonia	No
12	50	Unilateral	OS/C	NA	Mixed	2	2	Yes	Complete*	None	No	Cryotherapy + TTT + (M + T)	None	No
13	72	Bilateral	OD/D	B	Sphere	3	4	Yes	Complete	Severe pigment dispersion	Yes**	Nil	Nil	Nil

Ca, carboplatin; *f/up*, follow-up; *IAC*, intra-arterial chemotherapy; *IVC*, intra-venous chemotherapy; *Me*, melphalan; *(M + T)*, melphalan + topotecan injection; *OD*, right eye; *OS*, left eye; *RRD*, rhegmatogenous retinal detachment; *TTT*, transpupillary thermotherapy

*Vitreous seeds recurred after 4 months

**Enucleation was performed at the request of parents

†The tumor filled more than 50% of the vitreous volume

Table 2 Patients' demographics, clinical features, treatments, and outcomes in group with refractory vitreous seeds treated with intravitreal carboplatin plus melphalan

Case no	Age (month)	Laterality	Affected eye ICRB group	Other eye ICRB group	Previous and number of IViC injections	Type of vitreous seeds	Number of $(Ca+Me)^{+}$ injections	No. of affected quadrants	Subretinal seeds	Treatment response	Complications (6 months f/up)	Enucleation (6 months f/up)	Treatments used between 6 and 24 months of f/up	Complications (24 months f/up)	Enucleation (24 months f/up)
1	60	Unilateral	OS/D	NA	(M + T) × 3^{+}	Mixed	2	4	Yes	Partial	Hypotonia and cataract	Yes	Nil	Nil	Nil
2	48	Unilateral	OD/D	NA	(M + T) × 3	Mixed	5	2	No	Complete	RRD and recurrence	Yes	Nil	Nil	Nil
3	24	Unilateral	OS/D	NA	(M + T) × 4	Mixed	3	3	No	Complete	None	No	None	None	No
4	13	Bilateral	OD/C	D	(M + T) × 4	Dust	1	1	No	Complete	None	No	Cryotherapy + TTT	None	No
5	13	Bilateral	OS/D	A	(M) × 1	Dust	3	2	Yes	Complete	None	No	IAC + TTT	None	No
6	28	Unilateral	OD/C	NA	(M + T) × 4	Mixed	3	2	Yes	Complete	None	No	IVC	Hypotonia	Yes
7	36	Bilateral	OS/D	B	(M + T) × 3	Dust	2	2	No	Complete	None	No	IAC + TTT	Hypotonia	Yes
8	6	Unilateral	OD/D	NA	(M + T) × 2	Mixed	1	3	Yes	Complete	None	No	None	None	No
9	6	Bilateral	OS/C	D	(M + T) × 2	Dust	1	2	No	Complete*	None	No	(M + T) × 1	None	No

Ca, carboplatin; *f/up*, follow-up; *IAC*, intra-arterial chemotherapy; *IVC*, intra-venous chemotherapy; *Me*, melphalan; *(M + T)*, melphalan + topotecan injection; *OD*, right eye; *OS* left eye; *RRD*, rhegmatogenous retinal detachment; *TTT*, transpupillary thermotherapy

*Vitreous seeds recurred after 4 months

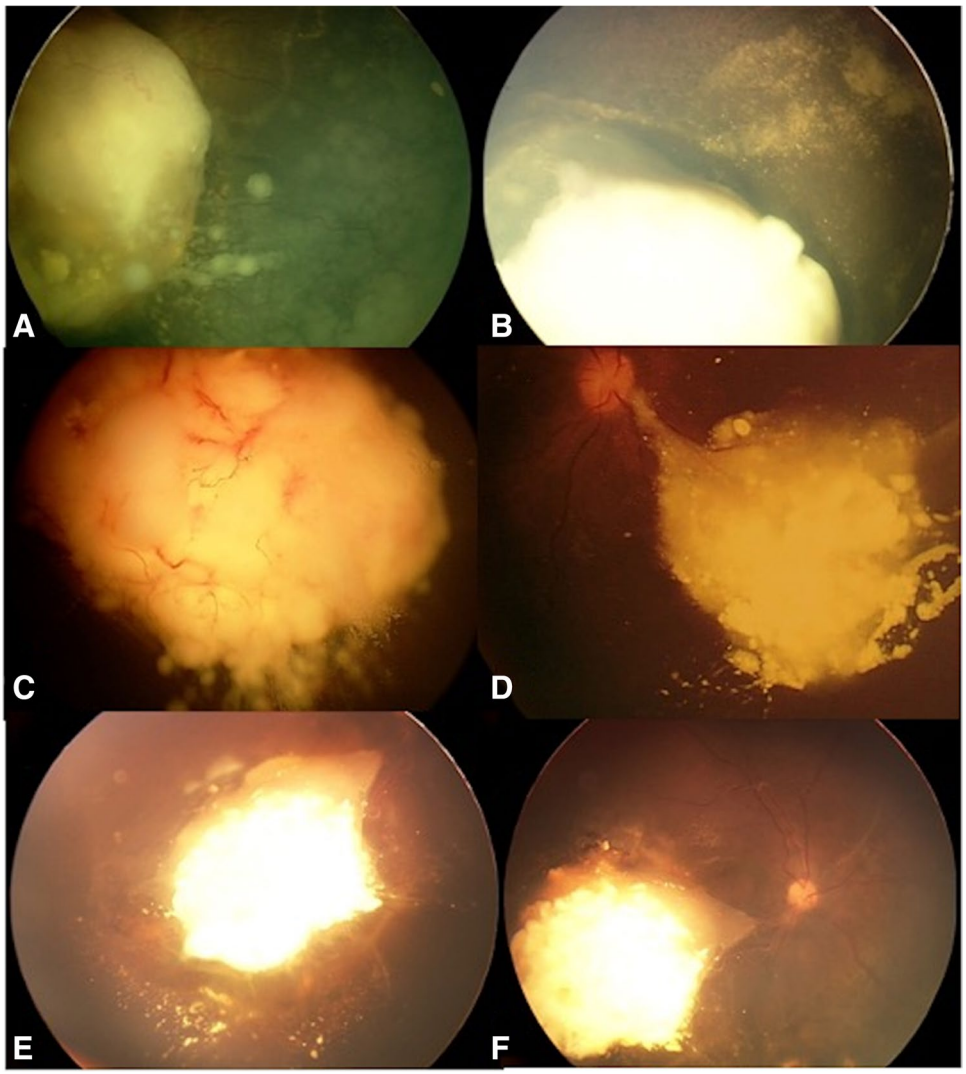

Fig. 1 Retinoblastoma vitreous seeds treatment using combined intravitreal carboplatin plus melphalan injection. **A** A group D unilateral retinoblastoma in a baby with unilateral involvement and a history of 3 cycles of intra-arterial chemotherapy (IAC). There was diffuse seeding inside the vitreous cavity. After 3 intravitreal injections of melphalan and carboplatin, there was resolution of all vitreous seeding (**B**). **C** A girl with group D unilateral RB had persistent tumor and diffuse seeding after 1 cycle of IAC. After treatment with 2 more cycles of IAC and 4 intravitreal injections of melphalan and carboplatin, there was resolution of all vitreous seeding and remarkable regression of the main tumor (**D**). **E** A girl with bilateral RB and group D in right eye. She had undergone 16 cycles of intravenous chemotherapy. At presentation, there was localized vitreous seeding that was managed with 2 intravitreal injections of melphalan and carboplatin. Six months after the last treatment, there were stable calcified vitreous seeds (**F**)

regression of vitreous seeds was only related to quadrant extension of vitreous seeds and those who responded most favorably demonstrated less than 3 quadrants of active vitreous seeds ($p=0.005$).

In this study, there was no relationship between the number of injections and vitreous seed extension (number of affected quadrants) ($p=0.25$), or type (e.g., dust, sphere, or mix-no sole cloud) of vitreous seeds ($p=0.50$). Complete vitreous seed regression did not correlate with the primary treatment (IVC, IAC, or both) ($p=0.45$) or regression type of the main tumor ($p=0.35$). Surprisingly, a total of 10 eyes had subretinal seeds before starting IVi (Ca-Me), which 8 of them regressing during the subsequent exams. None of these eyes received cryotherapy or TTT for the mentioned subretinal seeds before and during the regression.

Complications/toxicities following IVi (Ca-Me) injections during the 6-month follow-up period included retinal detachment ($n=1$ in both groups), early post-injection hypotony ($n=1$ in both groups), cataract ($n=1$ in the refractory group), and severe RPE dispersion ($n=1$ in the primary group). There was no sign of intraocular inflammation. Enucleation was necessary in 4 eyes ($n=2$ vs. $n=2$) for rhegmatogenous RD ($n=1$), phthisis bulbi ($n=2$), and tumor recurrence ($n=1$). Enucleation was performed in one patient at the request of the parents for personal travel reasons. In the long-term (24 months) follow-up period, hypotony was detected in 4 patients ($n=2$ in each group) and cataract in one eye of the primary group. Enucleation was necessary in 4 eyes ($n=2$ vs. $n=2$) for main tumor recurrence ($n=1$) and phthisis bulbi ($n=3$). Over 6-month follow-up from the date of initial IVi (Ca-Me), recurrent vitreous seeds were detected in 1 eye of each group in which managed successfully in both cases with intravitreal topotecan and melphalan. No additional recurrence of seeds was observed in any of the patients during the long-term follow-up period (24 months). There was no evidence of orbital invasion, systemic metastasis, or death.

Discussion

Despite significant advances in the treatment of RB, globe salvage in the context of vitreous seeding is still challenging and debatable [3, 6, 10]. In 1961, Ericson et al. introduced IViC to achieve a tumoricidal intraocular concentration of the chemotherapy agents to avoid systemic chemotherapy adverse effects [17].

Various studies have examined different IViC regimens for the treatment of vitreous seeds [1, 3, 6, 8, 18, 19]. Inomata and Kaneko investigated the in vitro susceptibility of RB tumors to 12 chemotherapeutic drugs and observed that tumor cells were most sensitive to melphalan [20]. After that, several studies were conducted to investigate the safety and efficacy of melphalan for RB vitreous seed control [3, 6, 8, 20]. According to studies, 6 to 8 intravitreal melphalan injections (as monotherapy) are required to manage vitreous seeds [3, 5]. Even though melphalan is the most commonly used drug in the treatment of RB vitreous seeds, the addition of a second chemotherapy agent to melphalan is inevitable for non-responding seeds [8]. However, there are still certain cases with resistant vitreous seeds even after combined therapy. It could be hypothesized that the second drug might be able to overcome the first agent resistance in some cases and lowering the doses of the agent [1]. On the other hand, some chemotherapy agents like topotecan may not be available in some developing countries. As a result, it is necessary to investigate the efficacy and safety of alternative medications for IViC.

Ghassemi et al. used combination of 40 µg of melphalan hydrochloride and 8 to 20 µg of topotecan hydrochloride and achieved 100% control of vitreous seeds with two mean injections. A total of 40 IViC was performed and a temporary hypotonia and vitreous hemorrhage and epithelial defect occurred in two cases. In a mean 15.2 months of follow-up, 67% of the 9 cases had no recurrences and 33% were enucleated. In the current study, 22 eyes with active primary or refractory (non-responsive or recurrent) vitreous seeds received 50 sessions of IVi (Ca-Me). Totally, IVi (Ca-Me) resolved vitreous seeds completely in 18 eyes with an average of 2 injections per eye during 6 months and then 24-month follow-up. In two cases, hypotonia and cataract and retinal detachment ensued. Enucleation was necessary in 4 eyes (18%) for rhegmatogenous RD, phthisis bulbi, and tumor recurrence. The combination treatment of IVi (Ca-Me) revealed complete vitreous seed control (3/4–4/5 of the study groups) over 6 months mean follow-up period. The vitreous seeds did not recur during the next 18 months, and 2 more enucleations were required in each group.

Clinical trials in animal models showed that repeated intravitreal carboplatin injections were safe [14, 15]. According to Harbour et al., intravitreal injections of carboplatin at doses of 10 µg or higher led to retinal toxicity in rabbit eyes, which have one fourth the vitreous volume of humans [22, 23]. Therefore, we chose carboplatin with a dose of 16 µg in 0.05 ml for intravitreal injections.

Hou et al. evaluated the efficacy of intravitreal injection of carboplatin (IViCa) plus bevacizumab every 4 weeks in 11 patients (11 eyes) with the diagnosis of refractory RB vitreous seeds by 3 months of follow-up. Seven patients exhibited a substantial reduction in vitreous seeds after 4 injections; however, control was poor in the other 4 cases, and recurrence of vitreous seeds was identified in 3 of them. They finally concluded that IViCa plus bevacizumab was partially effective and relatively safe for refractory vitreous seeds; however, other combinations of IViC were necessary [21].

A less frequent intravitreal injection could reduce the emotional and economic burden on the family, resulting in a better compliance with the therapy. Herein, we have demonstrated that IVi (Ca-Me) chemotherapy is a viable alternative for the treatment of primary and refractory vitreous seeds. In addition, we documented that IVi (Ca-Me) was effective for recalcitrant cases that failed previous intravitreal administration of melphalan alone or combined with topotecan. There was no significant statistical difference between primary and refractory groups in terms of complete vitreous seed regression.

Furthermore, we found that IVi (Ca-Me) had significant impact on subretinal seeds, and in eight of ten eyes with fundoscopy, subretinal seeds were evidently controlled. Although consolidation therapies (cryotherapy and/or TTT) were performed for solid tumors in 6 of the patients, we did not apply these adjuvant treatments on subretinal seeds. Similarly, Abramson et al. found that IViC with melphalan and/or topotecan was effective in treating subretinal seeds in about 87.6% of the eyes, with a low recurrence rate following a 10-month follow-up [24].

In vitreous seeding, the type of seed can affect the outcome. Francis et al. demonstrated that cloud seeds, compared to dust seeds, needed more injections to achieve full regression (median, 3 versus 8 injections), and that enucleation rates were more pronounced in the cloud seeds group [25]. Kiratli et al. revealed that the risk of enucleation was four times higher when the vitreous seed was mainly cloud-type [26]. In the present study, eyes with dust-type vitreous seeds responded more robustly to IVi (Ca-Me) than cloud type even though no significant relationship was found between the number of injections and the type of vitreous seeds ($p = 0.50$).

The reported side effects for intravitreal melphalan injection include visual loss, vitreous hemorrhage, RD, cataract, iris depigmentation, and chorioretinal atrophy [6, 18]. In limited published studies, intravitreal carboplatin injections have not

shown any specific side effects [13, 16, 21]. Due to the combination of melphalan and carboplatin, we could not observe any side effects related to carboplatin alone. It appears that intravitreal carboplatin is relatively safe with few noteworthy side effects. In this study, phthisis bulbi ($n=6$) and cataracts ($n=2$) were observed. Side effects may occur due to medications being delivered improperly near the ciliary body and lens and not at the center of the vitreous cavity, which can lead to ciliary body and lens toxicity. In addition, these eyes were related to the more advanced stages of the disease (group D or group E) and had undergone numerous and varied systemic or local treatments which could show more complications. Moreover, dose-dependent cataract formation or phthisis bulbi after intravitreal melphalan injections has been already reported [6]. In one study, about 30% of eyes with refractory or recurrent vitreous seeds developed cataract after multiple intravitreal 20–30 µg of melphalan injections [27]. In the present study, orbital invasion, systemic metastasis, and death were not observed during the 24-month follow-up period.

As a retrospective and nonrandomized study, this analysis contains some limitations including the small number of patients, lack of data concerning the visual function, and short follow-up period. Also, we included 4 cases of group E retinoblastoma ($n=4$ in the primary group and $n=0$ in the refractory group) that potentially could show more complications after treatments. Due to the absence of group E eyes in the refractory group, the outcome of this group is probably better than that in the primary group. Additionally, we only clinically investigated possible toxicity and objective functional evaluations like ERG were not available for these patients.

In conclusion, IVi (Ca-Me) may be another appropriate combined option for the treatment of primary and refractory vitreous seeds with few potential complications, especially in the situation of lack of access to topotecan. Additional comparative studies with longer follow-ups are warranted to establish the efficacy, optimum carboplatin dose, and interval of injections.

Acknowledgements Our oncology team is to be thanked for helping us complete this study successfully.

Data availability The data gathered and analyzed during the current study are available from the corresponding author. All the patients' data are provided in Tables 1 and 2.

Declarations

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional review board of Tehran University of Medical Sciences and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards (IRB Code: IR.TUMS.FARABIH.REC.1397.030).

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare no competing interests.

References

1. Ghassemi F, Shields CL, Ghadimi H et al (2014) Combined intravitreal melphalan and topotecan for refractory or recurrent vitreous seeding from retinoblastoma. JAMA Ophthalmol 132:936–941. https://doi.org/10.1001/jamaophthalmol.2014.414
2. Shields CL, Bianciotto CG, Jabbour P et al (2011) Intra-arterial chemotherapy for retinoblastoma: report no. 1, control of retinal tumors, subretinal seeds, and vitreous seeds. Arch Ophthalmol 129(11):1399–1406. https://doi.org/10.1001/archophthalmol.2011.150
3. Shields CL, Manjandavida FP, Arepalli S et al (2014) Intravitreal melphalan for persistent or recurrent retinoblastoma vitreous seeds: preliminary results. JAMA Ophthalmol 132:319–325. https://doi.org/10.1001/jamaophthalmol.2013.7666
4. Abramson DH, Marr BP, Dunkel IJ et al (2012) Intra-arterial chemotherapy for retinoblastoma in eyes with vitreous and/or subretinal seeding: 2-year results. Br J Ophthalmol 96:499–502. https://doi.org/10.1136/bjophthalmol-2011-300498
5. Manjandavida FP, Stathopoulos C, Zhang J et al (2019) Intra-arterial chemotherapy in retinoblastoma - a paradigm change. Indian J Ophthalmol 67:740–754. https://doi.org/10.4103/ijo.IJO_866_19
6. Ghassemi F, Shields CL (2012) Intravitreal melphalan for refractory or recurrent vitreous seeding from retinoblastoma. Arch Ophthalmol 130(10):1268–1271. https://doi.org/10.1001/archophthalmol.2012.1983
7. Kaneko A, Suzuki S (2003) Eye-preservation treatment of retinoblastoma with vitreous seeding. Jpn J Clin Oncol 33:601–607
8. Munier FL, Gaillard M-C, Balmer A et al (2012) Intravitreal chemotherapy for vitreous disease in retinoblastoma revisited: from prohibition to conditional indications. Br J Ophthalmol 96:1078–1083. https://doi.org/10.1136/bjophthalmol-2011-301450
9. Schaiquevich P, Carcaboso AM, Buitrago E et al (2014) Ocular pharmacology of topotecan and its activity in retinoblastoma. Retina 34:1719–1727. https://doi.org/10.1097/IAE.0000000000000253
10. Rao R, Honavar SG, Sharma V, Reddy VAP (2018) Intravitreal topotecan in the management of refractory and recurrent vitreous seeds in retinoblastoma. Br J Ophthalmol 102:490–495. https://doi.org/10.1136/bjophthalmol-2017-310641
11. Said AMA, Aly MG, Rashed HO, Rady AM (2018) Safety and efficacy of posterior sub-Tenon's carboplatin injection versus intravitreal melphalan therapy in the management of retinoblastoma with secondary vitreous seeds. Int J Ophthalmol 11:445–455. https://doi.org/10.18240/ijo.2018.03.15
12. Karkhaneh R, Pourmostadam B, Ghassemi F et al (2010) Toxic effect of intravitreal carboplatin detected by flattened electeroretinogram in a patient with retinoblastoma treated by chemoreduction and local treatments. Iran J Ophthalmol 22(3):57–60
13. Smith SJ, Pulido JS, Salomão DR et al (2012) Combined intravitreal and subconjunctival carboplatin for retinoblastoma with vitreous seeds. Br J Ophthalmol 96:1073–1077. https://doi.org/10.1136/bjophthalmol-2011-300829
14. Lemaître S, Poyer F, Fréneaux P et al (2020) Low retinal toxicity of intravitreal carboplatin associated with good retinal tumour control in transgenic murine retinoblastoma. Clin Experiment Ophthalmol 48:500–511. https://doi.org/10.1111/ceo.13711
15. Khodabande A, Ghassemi F, Asadi Amoli F et al (2021) Ocular safety of repeated intravitreal injections of carboplatin and digoxin: a preclinical study on the healthy rabbits. Pharmacol Res Perspect 9:e00814. https://doi.org/10.1002/prp2.814
16. Süsskind D, Hagemann U, Schrader M et al (2016) Toxic effects of melphalan, topotecan and carboplatin on retinal pigment epithelial cells. Acta Ophthalmol 94:471–478. https://doi.org/10.1111/aos.12990
17. Ericson L, Karlberg B, Rosengren BH (1964) Trials of intravitreal injections of chemotherapeutic agents in rabbits. Acta Ophthalmol 42:721–726. https://doi.org/10.1111/j.1755-3768.1964.tb01723.x

18. Manjandavida FP, Shields CL (2015) The role of intravitreal chemotherapy for retinoblastoma. Indian J Ophthalmol 63:141–145. https://doi.org/10.4103/0301-4738.154390
19. Shields CL, Douglass AM, Beggache M et al (2016) Intravitreous chemotherapy for active vitreous seeding from retinoblastoma: outcomes after 192 consecutive injections. The 2015 Howard Naquin Lecture. Retina 36:1184–1190. https://doi.org/10.1097/IAE.0000000000000903
20. Inomata M, Kaneko A (2004) In vitro chemosensitivity assays of retinoblastoma cells. Int J Clin Oncol 9:31–35. https://doi.org/10.1007/s10147-003-0370-4
21. Hou X, Cheng Y, Zhang Q et al (2015) Efficacy of intravitreal carboplatin plus bevacizumab in refractory retinoblastoma. Zhonghua Yan Ke Za Zhi 51:126–129
22. Harbour JW, Murray TG, Hamasaki D et al (1996) Local carboplatin therapy in transgenic murine retinoblastoma. Invest Ophthalmol Vis Sci 37:1892–1898
23. Friedrich S, Cheng YL, Saville B (1997) Drug distribution in the vitreous humor of the human eye: the effects of intravitreal injection position and volume. Curr Eye Res 16:663–669. https://doi.org/10.1076/ceyr.16.7.663.5061
24. Abramson DH, Ji X, Francis JH et al (2019) Intravitreal chemotherapy in retinoblastoma: expanded use beyond intravitreal seeds. Br J Ophthalmol 103:488–493. https://doi.org/10.1136/bjophthalmol-2018-312037
25. Francis JH, Abramson DH, Gaillard M-C et al (2015) The classification of vitreous seeds in retinoblastoma and response to intravitreal melphalan. Ophthalmology 122:1173–1179. https://doi.org/10.1016/j.ophtha.2015.01.017
26. Kiratli H, Koç I, Öztürk E et al (2020) Comparison of intravitreal melphalan with and without topotecan in the management of vitreous disease in retinoblastoma. Jpn J Ophthalmol 64:351–358. https://doi.org/10.1007/s10384-020-00743-2
27. Yousef YA, Al Jboor M, Mohammad M et al (2021) Safety and efficacy of intravitreal chemotherapy (melphalan) to treat vitreous seeds in retinoblastoma. Front Pharmacol 12:696787. https://doi.org/10.3389/fphar.2021.696787