Precision Intravitreal Chemotherapy for Localized Vitreous Seeding of Retinoblastoma

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Keywords

Retina · Retinoblastoma · Intravitreal chemotherapy · Melphalan · Precision injection · Retinal toxicity

Abstract

Purpose: To describe and evaluate the effectivity of a novel precision intravitreal injection technique for select cases of localized vitreous seeding in retinoblastoma. **Methods:** Patients with localized vitreous seeds from retinoblastoma received intravitreal chemotherapy via a precision injection technique (P-IVitC) intended to optimize local delivery of melphalan to target seeds. The needle was inserted trans pars plana and chemotherapy deposited within 3 mm of the target seed(s) under indirect ophthalmoscopic guidance. Without subsequent globe manipulation, the head was then positioned to promote gravity-dependent settling of chemotherapy. **Results:** There were 8 eyes of 8 patients treated with P-IVitC for active vitreous seeds following adequate solid tumor control with intravenous chemotherapy ($n = 3$, 38%) or intra-arterial chemotherapy ($n = 5$, 63%). Viable vitreous seeds were primarily solitary spheres ($n = 6$, 75%) and measured an average of 2.7 mm in diameter. Vitreous seed resolution ($n = 8$, 100%) was noted after a mean of 2.6 injections

(median 2; range 1–3) of 20 µg melphalan. There was no recurrence at a mean follow-up of 10.0 months. Only 1 eye had an associated complication (focal retinal pigment epithelial mottling, $n = 1$, 13%). **Conclusions:** With direct delivery of chemotherapy to within 3–4 mm of vitreous seed activity, P-IVitC provided complete control of localized vitreous seeds with minimal toxicity.

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Introduction

Intravitreal chemotherapy (IVitC) has revolutionized retinoblastoma vitreous seed management, achieving salvage rates of 87–100% for refractory vitreous seeding [1–7]. However, IVitC carries the risk of retinal toxicity [3–9], with retinal pigment epithelium (RPE) mottling occurring in 18–43% of treated eyes [2–6]. Francis et al. [7] found that each 30-µg melphalan injection resulted in a mean of 5.8 µV decrease in photopic electroretinogram response, often in the absence of RPE mottling [8]. Histopathologic studies of melphalan-exposed rabbit eyes revealed a spectrum of retinal damage, from mild thinning at a melphalan concentration of 5 µg/mL to severe at-

Fig. 1. Using the precision injection technique, the needle is inserted trans-pars plana. The injection site corresponds to the clock hour of vitreous seed burden. The needle is placed 3–4 mm (approx. 2.5–3 bevel lengths) of the vitreous seed under indirect ophthalmoscopic guidance and can reach all vitreous seeds at least 2.3 mm from the fovea. Chemotherapy is then delivered with the needle

bevel facing the seed. Without subsequent globe manipulation, the head is then positioned to assist in gravity-dependent settling of chemotherapy toward the target seed and away from the macula. Distance from the pars plana injection site to the fovea (19.0 mm) – standard needle length (0.5 in or 12.7 mm) – 4.0 mm margin to seed = 2.3 mm.

tenuation at 20 $\mu\text{g}/\text{mL}$ [8]. These insights suggest that retinal toxicity can be subclinical, dose-dependent, and indistinguishable even at low melphalan concentrations.

While the standard injection technique has been applied indiscriminately to all cases of vitreous seeding, a more localized delivery of chemotherapy could theoretically reduce the risk of diffuse retinal toxicity while still achieving tumoricidal concentrations at the targeted seeds. To our knowledge, no such technique for targeted intravitreal delivery has been reported despite 15–44% of vitreous seeding being localized to one quadrant [6, 9]. Herein, we describe a novel technique to target localized vitreous seeds with precision intravitreal chemotherapy (P-IVitC).

Methods

Data were retrospectively analyzed for patients receiving P-IVitC as salvage therapy for vitreous seeds between September 2013 and September 2017. This analysis adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of Wills Eye Hospital.

Each injection was performed under general anesthesia using a sterile technique. Melphalan was prepared in a manner previously described (20 μg melphalan in 0.1 mL diluent) and transferred to a sterile 1-mL syringe [4, 6]. The injection was performed using a standard 0.5-in 30-gauge needle inserted trans-pars plana at the clock hour coinciding with the epicenter of the vitreous seed burden in order to create the shortest path from injection site to vitreous seed location. Using indirect ophthalmoscopy, the vitreous seed size was estimated and chemotherapy was then injected with a needle bevel within 3–4 mm of the target vitreous seed(s) (Fig. 1).

Table 1. Precision intravitreal chemotherapy for retinoblastoma vitreous seeds: clinical features and treatment summary of 8 patients

Case No.	Findings at initial examination			Outcomes of previous therapy		Vitreous seed features			IVitC treatment		Vitreous seed outcome			
	age, mo/ sex/ laterality	ICRB group ^b	primary treatment (cycles)	solid tumor	vitreous seed status	interval to diagnosis ^c , mo	seed class	seed location ^d	melphalan dose, µg/0.1 mL	precision injections, <i>n</i>	seed response	time to response, mo	recur after IVitC	total follow-up, mo
1	57/F/OD	N/A	CRD (12)	Regress	Recur	22	Sphere	9/P	20	3	Resolve	0.5	No	4
2	4/F/OD	C	CRD (6)	Regress	Persist	0	Sphere	10/P	20	3	Resolve	0.9	No	26
3	29/F/OS	E	IAC (4)	Regress	Growth	7	Cloud	10/P	30 ^e	2	Resolve	0.5	No	2
4	32/M/OS	N/A	IAC (4)	Regress	Persist	0	Sphere	3/P	20	4	Regress	1.4	No	1
5	5/M/OS	D	IAC (6)	Regress	Growth	32	Sphere	5/A	20	4	Resolve	0.4	No ^f	17
6	9/M/OU ^a	D	IAC (5)	Regress	Growth	24	Mixed	5/A	20	3	Resolve	1.4	No	1
7	4/M/OU ^a	D	CRD (6)	Regress	Recur	29	Sphere	9/P	20	3	Resolve	1.4	No	17
8	33/M/OD	D	IAC (3)	Regress	Persist	0	Dust	9/P	20	4	Regress	1.0	No ^f	12

Class, classification; CRD, chemoreduction with intravenous vincristine, etoposide, and carboplatin, unless otherwise noted; F, female; Growth, appearance of new vitreous seed(s); IAC, intra-arterial chemotherapy; ICRB, International Classification of Retinoblastoma; IVitC, intravitreal chemotherapy; M, male; mo, months; N/A, not applicable; OD, right eye; OS, left eye; OU, both eyes; Persist, persistent vitreous seeds; Recur, recurrence of vitreous seeds; Regress, regression; RPE, retinal pigment epithelium. Resolution was defined as complete disappearance of vitreous seeds. Regression represented conversion of vitreous seeds to inactive remnants. Recurrence was defined as return to viability of previously regressed seeds. ^a Study eye was OD. ^b N/A if patient received treatment prior to our initial examination. ^c Time to diagnosis from initial examination (spontaneous seeds) or completion of previous therapy (recurrent seeds). ^d Clock hour of seed epicenter and anteroposterior (A/P) location within the globe with respect to the equator. ^e Melphalan was substituted for 30 µg/0.1 mL of topotecan due to possible melphalan allergy. ^f Enucleation was performed in the study eye for reasons other than vitreous seeding, including refractory neovascular glaucoma (patient 4) and refractory primary tumor (patient 8).

The length of the needle bevel (1.2 mm) was used to approximate the injection distance to the seed. Cryotherapy was applied at the scleral entry site as the needle was withdrawn. Triple freeze-thaw cryotherapy was completed with no subsequent globe manipulation. To optimize gravitational settling of chemotherapy, the patient's head was tilted toward the clock hour of the vitreous seed for approximately 45–60 min, which is the duration of time from injection to patient awakening in the postanesthesia care unit.

Patients were examined every 2 weeks during injections. After completion of the intravitreal therapy, follow-up intervals were extended based on vitreous seed response. Outcome measures included vitreous seed control and treatment complications. Follow-up duration was calculated from the date of the last intravitreal injection.

Results

This study included 8 eyes of 8 patients managed with P-IVitC for viable vitreous seeding. Demographic, classification, treatment, and outcome details are summarized in Table 1. Despite solid retinoblastoma regression ($n = 8$, 100%), all 8 eyes exhibited viable vitreous seed(s). Vitreous seeds persisted since the initial examination ($n = 3$, 38%), spontaneously appeared following treatment completion ($n = 3$, 38%), or represented tumor recurrence ($n = 2$, 25%). Vitreous seeds were solitary ($n = 6$, 75%) and classified as sphere ($n = 6$, 75%), cloud ($n = 1$, 13%), or a combination of dust, sphere, and cloud ($n = 1$, 13%). Average vitreous seed diameter measured 2.7 mm (median 2.0; range 0.5–5.0).

Mean patient age at initial P-IVitC was 37.8 months (median 35.7; range 14.2–61.1). The total number of in-

jection cycles were determined based on seed response. Each eye received a mean of 3.3 injections (median 3; range 2–4) of 20 µg melphalan, corresponding to a mean cumulative dose of 66 µg (median 60; range 40–80). Vitreous seed regression was noted after a mean of 2.6 (median 2; range 1–3) injections given over 0.9 months (median 1.0; range 0.4–1.4).

Following P-IVitC, all 8 (100%) cases demonstrated complete vitreous seed resolution without recurrence at a mean follow-up of 10.0 months (median, 8.0; range, 1.4–25.6) from the last injection (Fig. 2). Two study eyes were enucleated for reasons other than vitreous seeding. No eyes were enucleated secondary to refractory vitreous seeding.

Complications (RPE mottling at the site of melphalan injection) were noted in 1 (13%) case. The vitreous seed in that case was located 6 mm from the foveola, and RPE mottling spared the fovea by 3 mm (Fig. 2e–g).

Discussion

The P-IVitC technique is based primarily on fluid dynamics principles from computational modeling and in vitro experiments that show drug dispersion following intravitreal injection is largely driven by advective mass transport, such as natural saccadic movement, rather than molecular diffusion [10, 11]. In other words, injected drugs remain localized at the injection site unless perturbed by movements of the globe. Hence, P-IVitC differs from the standard IVitC approach in several key

Fig. 2. Clinical and optical coherence tomography (OCT) features of recurrent vitreous seeding before and after precision intravitreal chemotherapy (P-IVitC). Case 1: following completion of primary treatment, fundoscopy (**a**) and OCT (horizontal orientation, **b**) confirmed the presence of a parafoveal vitreous seed (white arrow). Three cycles of P-IVitC with intravitreal melphalan resulted in complete resolution of the seed (**c**) with intact fovea by OCT (**d**) and visual acuity of fix and follow. Case 2: following completion of primary treatment, fundoscopy (**e**) and OCT (horizontal orientation, **f**) disclosed a recurrent vitreous seed (white arrow) supero-

temporal and 6 mm from the foveola (yellow arrowhead), arising from previously treated retinoblastoma. After 3 cycles of P-IVitC melphalan, the vitreous seed (**g**) showed complete resolution. A linear pattern of RPE mottling (white arrowheads) was noted temporally, likely due to gravitational settling of injected melphalan, as the patient was rotated 45° onto the right ear with neck extension. Resolution of the vitreous seed was confirmed on OCT (**h**), exposing an underlying hyperreflective, calcified regressed scar. Visual acuity on 6-month follow-up was 20/50.

ways. First, instead of injecting centrally into the vitreous, chemotherapy is precisely injected near vitreous seed(s) under indirect ophthalmoscopic visualization. Second, the eye is not jiggled, eliminating the primary means of dispersion. Lastly, following injection, the head is positioned such that the target seed lies inferiorly, utilizing gravity to settle the denser melphalan (1.32 g/cm^3) toward the seed(s) within the vitreous (1.02 g/cm^3) and away from regions most critical for vision (e.g., macula). Gravitational settling of intravitreal medication and head positioning have previously been described [12].

The P-IVitC technique makes modifications to the standard injection technique, leveraging changes to the forces of dispersion to better localize delivery of intravitreal chemotherapy. These forces of dispersion can be likened to a droplet of ink within a glass of water. Advection describes the movement of ink particles along the bulk flow, such as when the water is stirred. Diffusion dictates the movement of ink particles from a high concentration to a low concentration. Lastly, if we presume the ink is denser than water, gravity will cause the ink to settle within the glass.

In this report, P-IVitC primarily targeted solitary spheres, with regression apparent after a mean of 2.6 injections compared to a mean of 4–5 injections reported previously [3, 4, 9]. Local retention of chemotherapy could expose target seeds to a higher concentration of active melphalan, accounting for the faster response. Larger studies are necessary to validate this suspected benefit.

Standard injections theoretically employ global dispersion, with a volume of distribution (V_d) equivalent to the entire pediatric vitreous humor (3.5 mL). Achieving a tumoricidal concentration of $4.0 \text{ } \mu\text{g/mL}$ for the entire vitreous gel would require a $14.4\text{-}\mu\text{g}$ melphalan dose ($3.5 \text{ mL} \times 4.0 \text{ } \mu\text{g/mL} = 14.4 \text{ } \mu\text{g}$) [13]. Ghassemi and Shields [14] corroborated this threshold clinically, observing that a melphalan dose of $8 \text{ } \mu\text{g}$ ($8 \text{ } \mu\text{g}/3.5 \text{ mL} = 2.3 \text{ } \mu\text{g/mL}$) was ineffective for seed control, while $50 \text{ } \mu\text{g}$ ($50 \text{ } \mu\text{g}/3.5 \text{ mL} = 14.3 \text{ } \mu\text{g/mL}$) proved to be effective but toxic. Others have balanced effectivity and toxicity at melphalan doses of $20\text{--}30 \text{ } \mu\text{g}$ ($5.7\text{--}8.6 \text{ } \mu\text{g/mL}$) [1, 4–6].

By localizing the chemotherapy, P-IVitC restricts the effective V_d , as melphalan can only diffuse so far before rendered inactive by its short 1-h half-life [15]. Willekens et al. [16] investigated intravitreal dispersion of iomeprol, a water-soluble contrast agent. Once injected into enucleated porcine eyes, iomeprol diffused through only 25% of the vitreous cavity (0.9 mL) in 1 h [16]. As-

suming comparable melphalan diffusion, a P-IVitC dose of just $3.5 \text{ } \mu\text{g}$ melphalan could theoretically reach the $4.0 \text{ } \mu\text{g/mL}$ tumoricidal concentration around target vitreous seed(s) ($0.9 \text{ mL accessible vitreous} \times 4.0 \text{ } \mu\text{g/mL tumoricidal concentration} = 3.5 \text{ } \mu\text{g dose}$). The effects of diffusion past 1 h could be clinically insignificant, owing to the short 1-h half-life of melphalan and increase in globe movements as the child awakens from anesthesia [15]. A 75% dose reduction to $3.5 \text{ } \mu\text{g}$ would likely reduce retinal toxicity, but its effectiveness in seed control would need to be tested.

In this report, RPE mottling was observed in 1 (13%) case. P-IVitC was performed for a vitreous seed located superotemporally (at 10:00) in the right eye, 6 mm from the foveola. The needle was inserted trans-pars plana at 10:00, corresponding to the seed clock hour. After the injection, the supine patient's head was rotated 45° toward his right ear with his neck slightly extended to promote gravitational settling of melphalan superotemporally, away from the macula. Mottling of the RPE was later observed, extending only 3 mm nasally from the seed, sparing the macula. This case highlights the unique precision afforded by P-IVitC but also raises the possibility that a lower melphalan dosage could have prevented RPE toxicity altogether.

There are limitations to our study and the P-IVitC technique. The small cohort and relatively short follow-up warrants further investigation of the therapeutic benefit and safety profile of P-IVitC for select cases of localized vitreous seeding. While there are published techniques to assess for extraocular dissemination of tumor cells following IVitC [17], these techniques were not used in our study given the presumably low risk associated with P-IVitC. Similar injection techniques have been safely employed for eyes with diffuse vitreous seeding. An analysis of 3,553 intravitreal chemotherapy injections for vitreous seeding of retinoblastoma revealed no extraocular dissemination, corresponding to a calculated risk of no greater than 0.08% [18]. Lastly, this technique's use of gravity has limitations for vitreous seeds in the superior and/or anterior aspects of the globe.

Treatment outcomes for vitreous seeding are measured by not only seed resolution, but also adverse ocular effects, particularly retinal and RPE toxicity. This report introduces a novel precision injection technique for localized vitreous seeding that theoretically exposes seeds to tumoricidal concentrations of chemotherapy while minimizing toxicity to healthy retina.

Acknowledgment

Support was provided in part by the Eye Tumor Research Foundation, Philadelphia, PA, USA (C.L.S.), the Lucille Weidman Fund for Pediatric Eye Cancer, Philadelphia PA, USA (C.L.S.), an unrestricted grant from Research to Prevent Blindness, Inc. (L.A.D.), the Heed Ophthalmic Foundation (L.A.D.), and a grant from the VitreoRetinal Surgery Foundation (L.A.D.). The funders had no role in the design and conduct of the study, in the collection, analysis and interpretation of the data, and in the preparation, review or approval of the manuscript. Carol L. Shields, MD has 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.

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Statement of Ethics

All procedures involving human participants were performed in accordance with the ethics standards of the Wills Eye Hospital Institutional Review Board and with tenets of the 1964 Helsinki declaration or comparable ethical standards.

Disclosure Statement

The authors have no conflicts of interest to disclose.