Published in final edited form as:

J Pediatr Ophthalmol Strabismus. 2016 ; 53(2): 96–98. doi:10.3928/01913913-20160311-01.

Cytopathological evaluation of ocular surface and needle washings following intravitreal melphalan injections for retinoblastoma

Jasmine H. Francis^{1,2}, Ijah Mondesire-Crump¹, Brian P. Marr^{1,2}, Scott E. Brodie^{1,3}, and David H. Abramson^{1,2}

¹Memorial Sloan Kettering Cancer Center, New York, NY, United States

²Weill Cornell Medical College, New York Presbyterian Hospital, New York, NY, United States

³Mount Sinai School of Medicine, New York, NY, United States

Abstract

There is concern that injections into retinoblastoma eyes are dangerous and allow tumor escapement. This study evaluated 200 ocular surface and 202 needle washings obtained from 280 injections into retinoblastoma eyes. With this injection and evaluation technique, no malignant cells were recovered, highlighting the low risk of the procedure.

Introduction

Historically, there has been hesitancy to inject into or aspirate through the wall of a retinoblastoma eye. The needle breaches the natural barrier of the eye and there is concern for exteriorization of tumor through the incision site. While the viability of these extraocular cells is unknown *in-vivo*, there are reports of retinoblastoma cells detected in the needle tract of enucleated eyes^{1,2}. Furthermore, tethering of vitreous seeds to the injection site of eyes treated with intravitreal melphalan has been reported³, highlighting the potential course of intraocular tumor towards the needle tract.

To better understand the risk of tumor externalization, our group analyzed the needle washings from retinoblastoma eyes receiving intravitreal melphalan. Our group has previously reported on the use of a safety precaution technique involving ocular water submersion following intravitreal injection⁴. We took this opportunity to also evaluate the ocular surface washings from the collected effluent for the presence of malignant cells.

Methods

This Institutional Review Board (IRB)-approved study included all eyes that received 30μg intravitreal melphalan and ocular surface or needle washing evaluation at Memorial Sloan-Kettering between Sept 2012 and April 2015. Informed consent was obtained for patients

from their guardian, caregiver or parent. Patient data included sex, laterality of disease, and age at initial intravitreal injection. Treatment data included mean number of injections per patient, number of injections with accompanying needle or ocular surface washing analysis and cytopathological results including presence of malignant, inflammatory, red blood or squamous cells.

The intravitreal injection and specimen collection were conducted as follows: the intraocular pressure was lowered to less than 10mmHg by digital massage; measuring 3 to 3.5mm posterior to the limbus, the intravitreal melphalan (30µg in 0.07cc) was injected with a 33-gauge needle. As the needle was withdrawn, the injection site was sealed and sterilized with cryotherapy (figure 1). The needle and syringe were sent to cytology to evaluate the presence of malignant cells. The ocular surface was submerged in irrigating sterile water for 3 minutes as previously described⁴ and the ocular surface effluent collected and sent to cytology (figure 2).

Results

The study included 280 injections, which were given to 63 eyes in 61 patients. 24 patients had unilateral disease and 37 had bilateral disease. 31 patients were male and 30 were female. The mean age at initial injection was 37.0 months and each patient received a mean of 5.3 total injections.

A total of 200 ocular surface washings and 202 needle washings were collected and sent to cytology for the presence of malignant cells. 74 injections had subsequent ocular surface washing analysis, 76 injections had subsequent needle washing analysis and 126 injections had both. All eyes receiving injections had active vitreous disease necessitating intravitreal melphalan.

Cytopathological results revealed no malignant cells in all 200 ocular surface and 202 needle washing specimens. In the ocular surface washings, there were squamous cells in 15 specimens, red blood cells (RBCs) in 2 specimens and inflammatory cells in 2 specimens. In the needle washings, there were squamous cells in 4 specimens, and no RBCs nor inflammatory cells in any specimen. At mean of 10.4 (range 0.5–28.3) months follow up, none of the eyes developed tumor growth at the needle site nor in the subconjunctival space. Two eyes developed seed tethering to the internal injection site³. There were no cases of metastatic disease nor deaths.

Discussion

The concern regarding extraocular dissemination of retinoblastoma through a needle tract has been reported in the literature. However, these reports predominantly describe diagnostic injections and employ a technique that differs from injecting therapeutics into the eye³. The majority of contaminated needle tracts occurred in instances where large gauge needles were used in an aspiration or biopsy technique to remove tissue from the eye. For instance, the 6 needles sites identified in 4 enucleation specimens, all had a diagnostic aspiration biopsy with a 25-gauge needle¹. In two other cases that succumbed to extraocular disease, one eye was biopsied⁵ and the other withstood a drainage procedure⁶. Despite these unfortunate

occurrences, invasive diagnostic procedures do not always yield unfavorable outcomes: in one cohort of 11 eyes that received vitrectomy for unsuspected retinoblastoma, none had histopathological evidence of tumor contaminating the vitrectomy port site⁷.

On the contrary, therapeutic injections involve placing fluid into the eye, without intentional aspiration and typically with smaller gauge needles. Despite this lower-risk procedure, there is literature to suggest that intraocular contents may course towards the injection site: as evidenced by vitreous incarceration following intravitreal injections⁸ and tethering of vitreous seeds to the injection site³. However, perhaps in part due to the adoption of safety-enhanced techniques, the proportion of eyes with extraocular extension following a therapeutic intravitreal injection is exceptionally low and calculated to be 0.007⁹. Ghassemi et al. confirmed this minimal risk, by examining the 30-gauge needle injection sites of 8 eyes that were enucleated following intravitreal melphalan and found no contamination by retinoblastoma cells. Of the 1304 eyes that received intravitreal injections and were reviewed by Smith et al., there were 2 cases of extraocular tumor attributable to a contaminated needle incision^{9,10}. Details of one case revealed “extraocular spread of retinoblastoma cells around the puncture site”, presumably with a 27-gauge needle and in a location with residual retinal tumor and vitreous seeds¹¹.

Safety enhanced techniques can be divided into those that prevent efflux of tumor cells (careful inspection of the injection site, cryotherapy to seal the site, reduction of intraocular pressure, needle gauge size and drug volume), and those that sterile escaped cells (cryotherapy, intravitreal or subconjunctival injection of drug and water submersion). The latter techniques may sterilize the needle tract, but fail to prevent efflux of tumor cells. This study evaluated the risk of these cells escaping the eye by examining the needle and ocular surface washings. We recovered no malignant cells from 200 ocular surface, nor 200 needle washing samples from retinoblastoma eyes that received intravitreal melphalan and were submerged in sterile water. This predicts with 95% certainty that no extraocular malignant cells would be found with this injection method and in the eyes injected. There are caveats to interpreting these findings: such as the possibility of not capturing effluxed cells that trap in the subconjunctival space or incomplete catching of tainted fluids draining from the eye. Furthermore, while the analysis of retinoblastoma by aspiration cytology is an accepted technique^{2,12–14}, this particular study lacked a positive control for its standard cytopathology assay.

In conclusion, it is reassuring to find an absence of cells outside the eye with the method described, and is in keeping with calculated data that suggests a low risk of extraocular extension following intravitreal injections. We welcome future studies to confirm our findings: the low risk of escaped retinoblastoma cells in injected eyes and the accompanying low risk of sequelae for the patient.

Acknowledgments

This work was support by the Fund for Ophthalmic Knowledge.

References

1. Karcioğlu ZA, Gordon RA, Karcioğlu GL. Tumor seeding in ocular fine needle aspiration biopsy. *Ophthalmology*. 1985; 92(12):1763–1767. [PubMed: 4088631]
2. Karcioğlu ZA. Fine needle aspiration biopsy (FNAB) for retinoblastoma. *Retina (Philadelphia, Pa)*. 2002; 22(6):707–710.
3. Francis JH, Marr BP, Brodie SE, Gobin YP, Abramson DH. Tethered vitreous seeds following intravitreal melphalan for retinoblastoma. *JAMA Ophthalmol*. 2014; 132(8):1024–1025. DOI: 10.1001/jamaophthalmol.2014.436 [PubMed: 25124953]
4. Francis JH, Xu XL, Gobin YP, Marr BP, Brodie SE, Abramson DH. Death by water: precautionary water submersion for intravitreal injection of retinoblastoma eyes. *Open Ophthalmol J*. 2014; 8:7–11. DOI: 10.2174/1874364101408010007 [PubMed: 24949111]
5. Goble RR, McKenzie J, Kingston JE, Plowman PN, Hungerford JL. Orbital recurrence of retinoblastoma successfully treated by combined therapy. *Br J Ophthalmol*. 1990; 74(2):97–98. [PubMed: 2310733]
6. Murthy R, Honavar SG, Vemuganti GK, Naik MN, Reddy VP. Systemic metastasis following hyphema drainage in an unsuspected retinoblastoma. *J Pediatr Ophthalmol Strabismus*. 2007; 44(2): 120–123. [PubMed: 17410964]
7. Shields CL, Honavar S, Shields JA, Demirci H, Meadows AT. Vitrectomy in eyes with unsuspected retinoblastoma. *Ophthalmology*. 2000; 107(12):2250–2255. [PubMed: 11097606]
8. Hodjatjalali K, Riazi M, Faghihi H, Khorami A. Ultrasound biomicroscopy study of vitreous incarceration subsequent to intravitreal injections. *Can J Ophthalmol*. 2012; 47(1):24–27. DOI: 10.1016/j.cjco.2011.12.013 [PubMed: 22333847]
9. Smith SJ, Smith BD. Evaluating the risk of extraocular tumour spread following intravitreal injection therapy for retinoblastoma: a systematic review. *Br J Ophthalmol*. 2013; doi: 10.1136/bjophthalmol-2013-303188
10. SS, AK. XVth Biannual Meeting, International Society of Ocular Oncology. Buenos Aires; Argentina: 2011. Vitreous injection therapy of melphalan for retinoblastoma. (Abstract)
11. Suzuki S, Aihara Y, Fujiwara M, Sano S, Kaneko A. Intravitreal injection of melphalan for intraocular retinoblastoma. *Jpn J Ophthalmol*. 2015; doi: 10.1007/s10384-015-0378-0
12. Sen S, Singha U, Kumar H, Ghose S, Sarkar T, Bajaj MS. Diagnostic intraocular fine-needle aspiration biopsy—An experience in three cases of retinoblastoma. *Diagn Cytopathol*. 1999; 21(5): 331–334. [PubMed: 10527480]
13. Walia R, Jain D, Mathur SR, Bakhshi S, Iyer VK. Metastatic retinoblastoma of mandible diagnosed on fine needle aspiration cytology. *Cytopathology*. 2014; 25(6):419–421. DOI: 10.1111/cyt.12115 [PubMed: 24750298]
14. Akhtar M, Ali MA, Sabbah R, Sackey K, Bakry M. Aspiration cytology of retinoblastoma: light and electron microscopic correlations. *Diagn Cytopathol*. 1988; 4(4):306–311. [PubMed: 3254808]

Figure 1.
Representative photograph of cryotherapy at the injection site as the needle is withdrawn from the eye.

Figure 2.
Representative photograph depicting water submersion and collection of ocular surface effluent.