Retinal Function and Corresponding Pathology in Advanced Retinoblastoma

Avery H. Weiss, MD; John P. Kelly, PhD; Raj P. Kapur, MD, PhD; Thomas Pendergrass, MD

Objective: To compare localized retinal function with corresponding histopathologic findings in advanced retinoblastoma.

Methods: The medical records and specimens of 7 children with Reese-Ellsworth stage V retinoblastoma (8 eyes) were retrospectively reviewed from January 1, 2005, through March 1, 2008. The patients underwent multifocal electroretinogram (mfERG) testing while imaging of the fundus was being performed. After enucleation of these eyes, retinal layers in a 10-mm-long section centered on the optic nerve were scored for histopathology.

Results: Visual acuity at presentation was 20/3000 to light perception in 6 of 6 eyes. Histopathologic analysis of the central retina revealed atrophy of all retinal layers in 4 eyes, moderate atrophy in 2 eyes, and mild atrophy of the outer retinal layers, respectively.

Conclusions: In advanced retinoblastoma, the mfERG amplitude provides a functional index of histopathologic retinal damage. When the retina is attached at presentation, the presence of a recordable mfERG indicates the potential for vision. When the retina is detached at presentation and reattaches after chemotherapy, the presence of a recordable mfERG also indicates the potential for limited vision. When the retina is detached or reattached, extinction of the mfERG is associated with severe retinal damage that may preclude visual recovery.

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Treatment of retinoblastoma is highly successful, with a cure rate in developed countries of approximately 95%.1,2 After tumor control, salvaging the globe and preserving vision are the major goals of current treatment strategies. Shields et al3 reported tumor control and globe salvage with chemotherapy and focal treatment in 85% of eyes with Reese-Ellsworth stages I through IV disease and 90% of eyes with International Classification of Retinoblastoma groups A, B, and C disease.4,5 In comparison, only half of the eyes with Reese-Ellsworth stage V or International Classification of Retinoblastoma group D retinoblastoma were salvaged with chemotherapy supplemented with focal therapy.6 Therefore, at least half of the eyes with Reese-Ellsworth stage V retinoblastoma require external beam irradiation, enucleation, or both.7 Given the potential complications of chemotherapy and irradiation,6,7 decisions regarding treatment of this group should include information about the potential for useful vision.

Visual outcomes after chemotherapy for Reese-Ellsworth stages I through V retinoblastoma have primarily been correlated with tumor extent and location. Although visual acuities are broadly distributed across these stages, better acuities are reported with smaller tumors that spare the macula, whereas worse acuities are noted with large tumors that involve the macula. Information regarding visual outcomes in patients with retinoblastoma stratified according to Reese-Ellsworth staging is limited. In this investigation, we measured retinal function in patients with Reese-Ellsworth stage V retinoblastoma who underwent enucleation. Our goal was to learn more about the ocular histopathologic features that underlie retinal function in these patients.

Methods

Institutional review board approval at Children's Hospital and Regional Medical Center, Seattle, Washington, was obtained for this study. The medical records and specimens from January 1, 2005, through March 1, 2008, were retrospectively reviewed. Seven patients underwent multifocal electroretinogram (mfERG) testing while using general anesthesia. Both pupils were preoperatively dilated with adult Kupfer solution. A fundus/eye camera stimulator (VERIS; EDI Inc, San Mateo, California) projected a scaled 103-hexagon pattern aligned with the optic disc. Flash intensity was 200 cd/m2. An electrode (ERG-Jet; Fabrinal SA, La Chaux-de-Fonds, Switzerland) was placed on the cornea, and signals were amplified 30 000 times and filtered 1 Hz to 1 kHz. The stimulator program presented a flashing hexagon pattern of 103 elements according to a pseudorandom binary sequence. The ERG was recorded after monitoring eye position with a fundus/eye camera stimulator and transilluminated by an infrared light source.

Author Affiliations: Divisions of Ophthalmology (Drs Weiss and Kelly), Pathology (Dr Kapur), and Oncology (Dr Pendergrass), Children's Hospital and Regional Medical Center, and Department of Ophthalmology, University of Washington Medical Center (Drs Weiss and Kelly), Seattle.
placed approximately 5 mm outside the lower eyelid margin or outer canthus (VERIS). Results were compared with the results of 2-year-olds’ control eyes recorded while the patient was anesthetized. Hexagons were flashed according to a pseudorandom binary m-sequence for a duration of $2^{14}$ flashes. Data were analyzed with a commercially available software program (VERIS 5.0). Tumor location was documented with a 60° fundus camera (RetCam; Massie Labs/Clarity Medical Systems (VERIS 5.0)). Tumor location was documented with a 60° fundus camera (RetCam; Massie Labs/Clarity Medical Systems (VERIS 5.0)).

Each enucleated globe was fixed in 10% formalin and sectioned serially at 0.5-cm intervals in either horizontal or parasagittal planes. The gross tissue samples were embedded in paraffin to obtain 4-µm histologic sections that were stained with hematoxylin-eosin. Histologic integrity of the neural retina was scored based on the severity of cell loss (atrophy), vacuolization, and gliosis in each of the 3 neural layers. Scoring extended from the edge of the optic disc to ±5 mm on each side in 1-mm intervals. The scoring criteria for each layer were as follows: 0, complete absence of neural retina; 1, marked atrophy with or without vacuolization; 2, moderate atrophy with or without vacuolization; 3, mild atrophy with or without vacuolization; and 4, no cellular loss. The optic nerve was selected because it provided a common and unequivocal reference point to align the sections, up to 30° of cyclorotation from the estimated plane section could occur. In addition, because of the nonlinear scaling of the mfERG hexagons, the stimulus slightly overlaps the histologic interval distance.

**RESULTS**

The Table summarizes the patient data. Six of 8 eyes were enucleated because of retinal detachment; the remaining 2 eyes were enucleated because of poor vision and vitreous seeding. Before enucleation, 6 of the 8 eyes were treated with chemotherapy only (5 eyes) or chemotherapy, external beam radiation, and laser therapy (1 eye; patient 3). Two eyes underwent primary enucleation. The interval from age at presentation to enucleation was 8 months or less in all but 1 patient. The mfERG testing was performed within 1 month of the initial examination in all but 1 patient. Visual acuities were severely reduced (20/3000 to light perception) in 6 of 8 patients with pretreatment measurements. Of the 2 patients without pretreatment visual acuity measurements, patient 3 had 20/400 visual acuity after treatment and reattachment and patient 6 was intolerant to monocular testing.

**Figure 1** A shows retinal images from the right eye of patient 3. The eye shows a large solid vascularized tumor and total retinal detachment. Figure 1B shows fundus photographs with superimposed mfERG responses in spatial registration with the retina. The mfERG responses were obtained after 6 courses of triple chemotherapy, external beam irradiation, and focal therapy. The retina reattached, and the regressed tumor is limited to the inferotemporal quadrant below the macula, but diffuse pigmentary disturbances exist throughout the posterior pole. The mfERG amplitude was subnormal throughout the 50° test area. The mfERG is extinguished in the epicenter of the tumor, but there are residual mfERG responses at the tumor perimeter and surrounding retina.

Figure 1C shows retinal images from the left eye of patient 2 at presentation. A 9 × 9-mm active tumor was centered temporal to the macula with a dense central aggregation of calcium. A string of vitreous seeds extended between the tumor and the optic disc. Figure 1D shows superimposed mfERG responses in spatial registration with the retina after completing 3 courses of triple chemotherapy. Compared with the normal mfERG results of the right eye, the affected eye responses were reduced in the area corresponding to the tumor. Residual function was present in the surrounding retina; however, amplitude was subnormal and latencies were delayed relative to the unaffected eye.

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**Table. Characteristics of the Study Patients**

<table>
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<th>Patient No.</th>
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<th>Enucleation</th>
<th>Fundus Findings</th>
<th>Retinal Status</th>
<th>Chemotherapy</th>
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</table>

Abbreviations: ERG, electroretinogram; ICR, the International Classification of Retinoblastoma; OD, right eye; OS, left eye; RD, total retinal detachment; ellipse, not known.

For an explanation of the ICR groups see Linn Murphree.®

All patients had Reese-Ellsworth stage V disease.

No visual acuity assessment before treatment. Visual acuity was 20/400 after chemotherapy and retinal reattachment.

Intolerant to monocular testing.
A representative histopathologic section from a 10-mm segment of retina flanking the optic disc of patient 3 is shown in Figure 2. The section corresponds to a horizontal plane that did not intersect the main tumor mass. It reveals a focus of tumor regression characterized by calcification and fibrosis of the choroid and absence of the overlying neural retina (Figure 2A). The remainder of the adjacent uninvolved retina shows variable degrees of neuronal loss, vacuolization, and reactive gliosis ranging from mild (Figure 2B) to severe (Figure 2G) for 1 or more retinal layers.

The correspondence between the mfERG and histologic scoring for 6 patients with a retinal detachment are summarized in Figure 3. In 3 of these 6 eyes, in which the retina was detached, the mfERG was extinguished (Figure 3, detached retinae). Histopathologic analyses in these 3 eyes revealed severe atrophy of all 3 layers or that the retina was replaced by tumor. In the remaining 3 eyes, which reattached after chemotherapy, the mfERG responses showed residual responses that corresponded with the severity of the histopathologic findings (Figure 3, reattached retinae). Patient 1 had an extinguished mfERG, and all retinal layers were severely atrophic. In patient 6, the histologic section transitioned from severe to moderate atrophy. The corresponding mfERG responses were severely distorted or extinguished in the region of severe atrophy, and a residual response was seen with subnormal amplitude in the region of moderate atrophy. Patient 3, whose retina reattached after chemotherapy, focal treatment, and external beam irradiation, showed moderate mfERG amplitude reductions. The corresponding histopathologic section shows moderate and mild atrophy in the photoreceptor layer and inner nuclear layer, respectively.

The correspondence between the mfERG and histologic scoring for 2 patients with attached retina is summarized in Figure 4. Patient 2, who had the mildest histopathologic changes in the series, showed only mild amplitude reduction and latency prolongation compared with the unaffected eye. In patient 5 with limited...
retinal tumor but dense vitreous seeding and opacification, mfERG amplitudes were relatively large, but latencies were severely delayed. The observed latency delays are likely related to the moderate histopathologic abnormalities noted in the retina and attenuation of stimulus intensity by the vitreous opacification.

**Figure 2.** Histologic scoring of retinal pathology segments (hematoxylin-eosin). A section through the enucleated eye of patient 3 (center panel) includes 5-mm-long strips of retina on either side of the optic disc. Each strip was subdivided into 1-mm segments (A-J), which were scored as described (see “Methods” section). Representative images of the neural retina in each segment are shown at identical magnification in panels A through J (scale bar in J), and the scores for the 3 retinal cell layers are provided in the table at the bottom of the figure.
This study provided objective assessment of retinal function in children with Reese-Ellsworth stage V retinoblastoma. We found that the mfERG reflects the corresponding histopathologic findings within the central 50° of the retina. The presence of a recordable mfERG indicated preservation of the neurosensory retina, especially the photoreceptors and the potential for useful vision. The high correspondence between the mfERG and corresponding histopathologic findings with retinal reattachment after chemotherapy was particularly informative. Severe reduction or extinction of the mfERG response suggests that the retina is replaced by tumor or is severely atrophic and that the severity of retinal abnormalities may preclude recovery of vision.

Half of the eyes in this study had extinguished mfERG responses. Extinction of the mfERG can arise from selective loss of cone photoreceptors; however, the corresponding histopathologic sections showed severe atrophy of all retinal layers or replacement of the neural retina by tumor. In our series, histopathologic evidence of neuronal loss and gliosis without associated tumor involvement are consistent with prolonged hypoxemia associated with chronic retinal detachment. In support of this notion, 3 of these patients had retinal detachment at the time of the mfERG. Our data have shown that cone density amplitudes are significantly reduced in detached areas but are still recordable. Therefore, the finding of an extinguished mfERG in a detached retina suggests that there is severe retinal atrophy because of the extended duration of retinal detachment or complete retinal replacement by tumor. Even with reattachment of the retina after chemotherapy, areas without residual tumor showed generalized retinal atrophy and an extinguished mfERG. Retinal detachment probably exists for an extended period owing to delays in the diagnosis of retinoblastoma. Prior studies indicate that as little as a week or more of retinal detachment can lead to irreversible visual acuity and photoreceptor damage and that recovery of visual function diminishes exponentially with increased duration of detachment. Shields et al reported final visual acuity of 20/50 and 20/70 after reattachment of the retina in a patient with bilateral stage V retinoblastoma treated with chemotherapy. Although our sample size is limited, the histopathologic findings of severe retinal atrophy and retinal function loss indicate that a subset of patients with stage V disease and retinal reattachment is unlikely to recover significant vision.

The remaining 50% of eyes in this study retained some mfERG response. The residual mfERG response is consis-
tent with partial preservation of the photoreceptors and inner nuclear layers, which are the primary generators of the mfERG. Although present, the waveforms of residual mfERG responses were abnormally prolonged and their amplitudes were reduced (except in patient 5). In addition, we observed areas of residual mfERG response superimposed on some tumor locations, suggesting there is functioning retina in these regions. However, a residual mfERG response in these sites could be related to signal averaging across unaffected and affected regions of the retina. In areas with disproportionate loss in the photoreceptor layer, the mfERG amplitude is more reduced. We cannot exclude the possibility that a residual response is due to light reflection from the white surface of the calcified tumor that stimulates unaffected retina rather than the intended location.

Retinal toxic effects due to chemotherapy may contribute to the observed mfERG reductions. Vincristine is implicated in retinal toxic effects.14 Vincristine disrupts microtubule-mediated intracellular transport and reduces synaptic efficiency between the photoreceptors and secondary neurons, which leads to a reduced ERG b wave. Carboplatin can result in suppression of the ERG b wave and loss of inner retinal neurons, including photoreceptor damage, but only at higher toxic levels.15 Our mfERG data do not show selective loss of the b wave, indicating a generalized reduction in retinal function unrelated to chemotherapy. To our knowledge, external beam irradiation is below the threshold associated with retinal damage.

In unilateral Reese-Ellsworth stage V retinoblastoma, the benefits of salvaging an eye with poor acuity due to loss in retinal function and superimposed amblyopia must be weighed against the potential short- and long-term complications of chemotherapy. All patients in this study had severe reductions in visual acuity at the time of diagnosis. Loss of acuity is primarily attributed to extensive tumor of the retina, macular involvement, retinal detachment, or opacification of the ocular media. In each of these scenarios, particularly in unilateral or asymmetric disease, there is likely superimposed amblyopia. When the mfERG reveals large regions of preserved retinal function, the argument for chemotherapy and potential salvage of the eye is stronger. In comparison, when the mfERG is severely reduced or extinguished, the major benefit of chemotherapy is to preserve an eye with severely reduced vision. Although most patients tolerate the chemotherapy, a significant percentage experience short-term bone marrow suppression with cytopenia, neutropenia with concurrent infections, vincristine neurotoxic effects, gastrointestinal symptoms, and dehydration. The major long-term concern is a higher prevalence of acute leukemia after treatment with etoposide.1

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Correspondence: Avery H. Weiss, MD, W-4753/Children’s Hospital and Regional Medical Center, 4800 Sand Point Way NE, Seattle, WA 98105.

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REFERENCES