Histopathologic Findings in Eyes With Retinoblastoma Treated Only With Chemoreduction

Hakan Demirci, MD; Ralph C. Eagle, Jr, MD; Carol L. Shields, MD; Jerry A. Shields, MD

Objective: To evaluate the histopathologic findings in the eyes with retinoblastoma that had been treated only with chemoreduction.

Design: Clinicopathologic series.

Study Material: Ten eyes of 8 patients with retinoblastoma that were enucleated after therapy consisting only of systemic chemotherapy (chemoreduction).

Methods: All cases received a chemoreduction regimen including a combination of intravenous carboplatin, etoposide phosphate, and vincristine sulfate. Adjuvant treatment to the tumor was not provided in any case. The enucleated globes were studied by routine light microscopy.

Main Outcome Measure: Histopathologic features of retinoblastoma following chemoreduction.

Results: At presentation, there were 8 eyes in Reese-Ellsworth group V, 1 eye in group IV, and 1 eye in group III. After chemoreduction (mean, 4 cycles; range, 1-6 cycles), the main tumor regressed a mean 34% in thickness and 24% in basal diameter. The indication for enucleation was retinoblastoma recurrence as subretinal and/or vitreous seeds in 7 eyes and extensive vitreous hemorrhage in 3 with uncertainty about viable-appearing tumor. In no case was enucleation performed for recurrence of the main tumor. In all eyes, there was histopathologic evidence of tumor regression. In 8 of 10 eyes, histopathologic examination disclosed tumor regression without viable-appearing retinoblastoma in the main tumor. Of these 8 eyes, 2 showed a completely calcified glial scar and 6 showed an apical calcified glial scar and a basal residual well-differentiated component with retinomalic and/or retinocytomalike features. In the remaining 2 eyes, an area of posttherapeutic regression was present but contained foci of mitotically active, viable-appearing malignant retinoblastoma cells. The 6 eyes found to contain well-differentiated component with retinomalic and/or retinocytomalike features showed a mean decrease of 17% in largest basal dimension and 32% in thickness after a mean of 3 cycles of chemoreduction. In contrast, the 4 eyes that did not contain well-differentiated component with retinomalic and/or retinocytomalike features showed a mean decrease of 35% in largest basal dimension and 55% in thickness after a mean of 5 cycles of chemoreduction. Of those 7 eyes enucleated for recurrent subretinal and/or vitreous seeds, viable tumor seeds were confirmed histopathologically in all cases. There was no histopathologic evidence of chemotherapeutic toxicity to the eye.

Conclusions: Histopathologic examination of 10 enucleated eyes following chemoreduction alone revealed that the main retinoblastoma regressed in all eyes. Additionally 6 eyes showed basal residual well-differentiated component with retinomalic and/or retinocytomalike features, and these eyes also displayed less shrinkage with chemoreduction. Despite the lack of viable-appearing retinoblastoma within the main tumor, enucleation was performed for viable subretinal and/or vitreous seeds in 7 cases and confirmed histopathologically.

Arch Ophthalmol. 2003;121:1125-1131
There is limited information about the histopathologic findings in eyes with retinoblastoma following chemotherapy.12,13 Bechakis et al12 correlated clinical regression patterns with histopathologic features in 5 eyes with retinoblastoma previously treated with chemoreduction. Subsequently, Dithmar et al13 reported the histopathologic findings of clinical type 3 regression pattern in 2 eyes with retinoblastoma following chemoreduction. To better understand the effects of chemoreduction, we retrospectively evaluated 10 eyes with retinoblastoma that were enucleated after therapy consisting only of chemoreduction.

METHODS

All patients with retinoblastoma who were treated with chemoreduction on the Ocular Oncology Service at Wills Eye Hospital, Thomas Jefferson University, Philadelphia, Pa, between July 1, 1996, and September 30, 2000, were identified. Those patients who (1) were treated with chemoreduction, (2) received no direct tumor treatment such as laser therapy, cryotherapy, transpupillary thermotherapy, or plaque radiotherapy during or after chemoreduction, and (3) came to enucleation were included in this study.

Patient data were reviewed and demographic information, clinical findings, and chemotherapeutic parameters were tabulated. Patient data collected on the initial visit to the Ocular Oncology Service included age at diagnosis (months), race (African American, Hispanic, Asian, or white), sex (female or male), hereditary pattern (familial or sporadic), laterality (unilateral or bilateral), and the involved eye (right or left). The clinical information recorded on the initial visit included Reese-Ellsworth classification, meridional location (superior, superonasal, nasal, inferonasal, inferior, inferotemporal, temporal, superotemporal, macula, or diffuse), basal dimensions of main tumor (in millimeters), thickness of main tumor (in millimeters), and the presence of retinal detachment, subretinal tumor seeds, or vitreous tumor seeds. Additional clinical information recorded at the time of enucleation included basal dimensions of main tumor (in millimeters); thickness of main tumor (in millimeters); the presence of retinal detachment, subretinal tumor seeds, or vitreous tumor seeds. Additional clinical information recorded at the time of enucleation included basal dimensions of main tumor (in millimeters); thickness of main tumor (in millimeters); the presence of retinal detachment, subretinal tumor seeds, or vitreous tumor seeds; and the reason for enucleation.

The drugs and chemotherapeutic protocol used were identical to that published in our previous studies and included a combination of intravenous carboplatin, etoposide phosphate, and vincristine sulfate.3,7-11 Initially, all patients were scheduled to receive 6 cycles of chemotherapy. In those instances in which 6 cycles were not administered prior to enucleation, the number of cycles actually administered was recorded.

All enucleated eyes were fixed in 10% neutral buffered formalin, embedded in paraffin, and processed routinely for light microscopy. Representative microscopic sections prepared from the pupil–optic nerve block containing the largest part of the tumor and all additional segments of tumor-bearing tissue that had been submitted for histopathologic examination were retrieved from laboratory archives and reviewed by an experienced ophthalmic pathologist (R.C.E.) who had no knowledge of treatment. During examination, tumor dimensions were measured with millimeter calipers or an ocular micrometer.

The assessed histopathologic parameters focused on the status of retinoblastoma regression classified as (1) viable-appearing retinoblastoma, (2) regressed retinoblastoma, or (3) regressed retinoblastoma with well-differentiated component with retinomalike and/or retinocytomalike features. Histopathologic criteria for viable-appearing retinoblastoma included an infiltrate composed of poorly differentiated, mitotically active, and focally necrotic tumor cells with hyperchromatic nuclei and scanty cytoplasm. Criteria for regressed retinoblastoma included replacement of the tumor by a glial scar containing characteristic foci of calcified tumor cells. Criteria for regressed retinoblastoma with well-differentiated component with retinomalike and/or retinocytomalike features included a tumor composed of cells with abundant cytoplasm that appeared relatively paucicellular and eosinophilic compared with retinoblastoma. Additional features included bland nuclei, absence of mitotic figures, presence of photoreceptor differentiation, and foci of calcification within viable areas of the tumor. The sectioned eyes were also evaluated for subretinal tumor seeds (present or absent, and location), vitreous seeds (present or absent), choroidal invasion (absent, microscopic, or macroscopic), optic nerve invasion (absent, prelaminar, laminar, or postlaminar), and findings consistent with chemotherapeutic toxicity in the retina or choroid (present or absent).

RESULTS

Of 252 patients with retinoblastomas who were primarily managed on the Ocular Oncology Service at Wills Eye Hospital between July 1, 1996, and September 30, 2000, 203 eyes of 133 patients were treated with chemoreduction as part of their management. Ten eyes (5%) of 8 patients (6%) who were treated with chemoreduction alone were subsequently enucleated and form the basis for this study (Table 1).

Clinical data at the initial visit, indications for enucleation, and clinical data prior to enucleation are given in Table 1. At the initial visit, the mean largest basal diameter of the main tumor was 20 mm (median, 20 mm; range, 15-24 mm) and the mean thickness was 10 mm (median, 9 mm; range, 5-16 mm). After a mean of 4 cycles of chemotherapy (median, 4 cycles; range, 1-6 cycles), the main tumor showed a mean decrease of 25% in the largest basal diameter (median, 24%; range, 8%-40%) and a mean decrease of 42% in thickness (median, 47%; range, 8%-77%). The average interval between the initiation of chemotherapy and enucleation was 7 months (median, 4 months; range, 1-36 months). In no case was enucleation performed for recurrence of main tumor (Table 1).

The cases were classified into 3 groups (viable-appearing retinoblastoma, regressed retinoblastoma, and regressed retinoblastoma with well-differentiated component with retinomalike and/or retinocytomalike features) on the basis of histopathologic findings. The latter are summarized in Table 2. Overall, all eyes showed retinoblastoma regression. In 8 patients (case 1, case 2, right eye; cases 4-6, 7, right and left eyes; and case 8) regression was complete (Figure 1). Two of the retinoblastomas were totally replaced by calcified glial scar (case 1 and case 2, right eye), in the remaining 6 cases, an apical calcified glial scar was found on the inner surface of a basal well-differentiated component with retinomalike and/or retinocytomalike features (cases 4-6, 7, right and left eyes, and case 8) (Figure 2). In some eyes the areas of residual well-differentiated component with retinomalike and/or retinocytomalike features were extensive measuring up to 18 mm in diameter and 8 mm in
thickness (Figure 3). In 2 cases (case 2, left eye, and case 3), partial regression with residual apparently viable retinoblastoma was present.

At the initial visit, when assessing only those 6 eyes with residual well-differentiated component with retinomalous and/or retinocytomalous features, the mean largest basal diameter of the main tumor was 20 mm (median, 20 mm; range, 17-24 mm) and the mean thickness was 9 mm (median, 9 mm; range, 8-14 mm). After a mean of 3 cycles of chemoreduction (median, 3 cycles; range,
1-6 cycles), the largest basal dimension of main tumor decreased only 17% in mean basal diameter (median, 17%; range, 8%-29%) and 32% in mean thickness (median, 34%; range, 8%-50%). The average interval between the initiation of chemotherapy and enucleation was only 3 months (median, 3 months; range, 1-6 months) in this group of patients.

At the initial visit, when assessing only those 4 eyes found later to contain no evidence of residual well-differentiated component with retinomalike and/or retinocytomalike features, the mean largest basal diameter of the main tumor was 20 mm (median, 20 mm; range, 15-24 mm) and the mean thickness was 12 mm (median, 12 mm; range, 8-16 mm). After a mean of 5 cycles of chemoreduction (median, 6 cycles; range, 4-6 cycles), the largest basal diameter of the main tumor decreased 35% in mean basal diameter (median, 38%; range, 25%-40%) and 55% in mean thickness (median, 53%; range, 44%-70%) (Figures 1B and 2B). The average interval between the initiation of chemotherapy and enucleation was 14 months (median, 6 months; range, 4-36 months).

In 7 eyes, subretinal seeds of residual or recurrent retinoblastoma were observed adjacent to the main mass or in the peripheral retina. The subretinal seeds were composed of poorly differentiated, mitotically active, baso-
philic retinoblastoma cells (Table 2). There was no microscopic or macroscopic choroidal invasion and no degree of optic nerve invasion in any eye. No histopathologic findings that could be attributed to chemotherapeutic toxicity were observed.

In the 2 eyes, which showed a type 1 regression pattern following 4 and 6 cycles of chemoreduction, histopathologic examination disclosed total regression and a focally calcified glial scar (Table 2). There were no viable-appearing retinoblastoma cells in these 2 eyes. The other 8 eyes showed type 3 clinical regression patterns. In addition to regressed tumor, 2 of the 8 eyes contained viable-appearing retinoblastoma and 6 contained well-differentiated component with retinoma-like and/or retinocytomalike features. Tumors with areas of well-differentiated component with retinoma-like and/or retinocytomalike feature had a classic fish-flesh appearance.

COMMENT

Chemotherapy has an important role in the management of intraocular retinoblastoma.1,2,14 In a pilot study, Shields et al7 reported a dramatic response to 2 cycles of a combination of vincristine, etoposide, and carboplatin with a 49% decrease in tumor thickness, a 35% decrease in basal diameter, and complete resolution of subretinal fluid in 76% of the patients. Murphree et al5 studied 35 eyes with retinoblastoma and showed that chemoreduction with focal therapy was successful in all 10 eyes in Reese-Ellsworth groups I through IV but was unsuccessful in all 7 eyes that had extensive subretinal seeding and all 18 eyes with group Vb tumors with vitreous seeding, which were treated with external beam radiotherapy or enucleation. Gallie et al4 observed that the tumor control rate increased significantly when cyclosporin was added to the treatment regimen. In a larger group of eyes treated with 6 cycles of vincristine, etoposide, and carboplatin and adjuvant treatment, Shields et al10,11 found that chemoreduction and focal conservative treatment for retinoblastoma was effective, but recurrence of at least 1 retinal tumor, vitreous seed, or subretinal seed occurred in approximately 50% of the eyes by 5-year follow-up. The globe salvage rate was 85% for eyes classified as having Reese-Ellsworth groups I through IV and 47% for Reese-Ellsworth group V by 5-year follow-up.11

Despite the recent enthusiasm about chemoreduction, there is relatively little reported about the ocular histopathologic features of treated cases. Bechrakis et al12 examined 5 eyes with retinoblastoma that received 2 to 5 cycles of chemoreduction and they confirmed the efficacy of chemoreduction histopathologically and correlated the histopathologic findings with clinical regression patterns. In eyes with type 1 regression pattern,12 they observed that the tumor was completely replaced by reactive gliosis, calcification, and necrosis without viable-appearing cells. In type 2 regression pattern,11 well-differentiated viable-appearing retinoblastoma cells were observed surrounding the central necrotic area. In type 3 regression pattern,13 areas of gliosis, calcification, and necrosis and areas of purportedly nonviable retinoblastoma cells showing moderate differentiation were seen. Subsequently, Dithmar et al13 reported a case with type 3 regression pattern that contained a gliotic mass with scattered calcification and no residual retinoblastoma after 2 cycles of chemoreduction and another case with type 3 regression pattern that contained both viable-appearing retinoblastoma cells and a gliotic, calcified scar after 1 cycle of chemoreduction. They proposed that chemoreduction had variable effects on retinoblastoma.

In this study, we have found a correlation between clinical regression patterns following chemoreduction and histopathologic findings. In the 2 eyes in our series with
clinical type 1 regression pattern, the retinoblastoma had completely regressed with findings of necrosis and a glial scar that contained characteristic foci of calcification. No viable-appearing retinoblastoma cells were observed in these 2 eyes. Histopathologic examination of the other 8 eyes with type 3 clinical regression patterns disclosed a biphasic pattern in the main tumor. The latter was marked by an area of posttreatment regression and a second area of viable-appearing tumor that was classified as malignant in 2 eyes and benign in 6 eyes. In the 2 eyes with malignant residua, the secondary component was composed of mitotically active, unequivocally malignant retinoblastoma cells that had basophilic nuclei, scant cytoplasm, and formed perivascular sleeves. In the remaining 6 eyes with benign tumor residua, this component, which invariably formed the basal part of the tumor, was well differentiated and appeared cytologically bland without mitotic activity. Compared with the viable-appearing parts of a typical malignant retinoblastoma, such areas were relatively eosinophilic and contained photoreceptor differentiation (fleurettes). In addition, they contained foci of calcification that were located within viable-appearing parts of the tumor. All of the previously mentioned were characteristic histopathologic features of benign retinoma or retinocytoma.

These extensive areas of residual well-differentiated component with retinomalike and/or retinocytomalike features were one of the most distinctive observations in this study. Bland well-differentiated component with retinomalike and/or retinocytomalike features were found in 6 of 10 eyes and invariably constituted the basal part of a biphasic tumor. In several patients, the residual areas of well-differentiated component with retinomalike and/or retinocytomalike features were extensive, the largest measuring 18 mm in diameter and 8 mm in thickness.

The main reason for enucleation in this group of patients was vitreous hemorrhage and tumor recurrence as subretinal and/or vitreous seeds. Subretinal and vitreous seeds have no blood supply and obtain nutrients through the subretinal fluid or vitreous cavity. The viability and recurrence of such tumor seeds may be related to the poor penetration of systemic chemotherapeutic agents.

The degree of cellular differentiation of retinoblastoma cells can affect the clinical response to chemoreduction. Ts'o et al reviewed 42 eyes with retinoblastoma that had been enucleated because they responded poorly to radiotherapy and found that 17 (40%) contained foci of photoreceptor differentiation. In contrast, they found photoreceptor differentiation in only 18 (6%) of 300 eyes that had been enucleated primarily without prior radiotherapy. They speculated that highly differentiated components of retinoblastoma were comparatively resistant to irradiation, while the mitotically active, undifferentiated components were sensitive to irradiation and appeared regressed. Carbboplatin, vincristine, and etoposide are radiomimetic agents that damage the fundamental mechanisms concerned with cell proliferation, particularly DNA synthesis and cell division. It is probable that the cells of well-differentiated retinal tumors would show similar resistance to chemotherapy. In vitro drug studies using different retinoblastoma cell lines have shown that undifferentiated retinoblastoma cells are more sensitive to carboplatin than differentiated retinoblastoma cells. Similarly, we have previously observed a lack of clinical response to chemoreduction in 2 eyes presumed to contain well-differentiated retinal tumors. On the other hand, one cannot totally exclude the possibility that chemoreduction might induce differentiation in retinoblastoma.

In this study, the indications for enucleation were retinoblastoma recurrence as subretinal and/or vitreous seeds and extensive vitreous hemorrhage. In no case was enucleation performed for recurrence of the main tumor. This may cause bias in our findings. Because of the few eyes enrolled in this study, the conclusions might not always be generalized to the overall population of patients with retinoblastoma.

Histopathologically, we have observed that chemoreduction alone seems to be effective for some cases of retinoblastoma but subretinal and vitreous seeds may not respond completely. A regimen consisting solely of chemoreduction induced varying degrees of clinical and histopathologic regression in the 10 examined eyes. Additionally, 6 of 10 eyes harbored biphasic lesions composed of an area of tumor regression on the inner surface of a focus of residual well-differentiated component with retinomalike and/or retinocytomalike features. Eyes found to harbor such well-differentiated component with retinomalike and/or retinocytomalike features showed less shrinkage from chemoreduction.

Submitted for publication October 10, 2002; final revision received March 24, 2003; accepted April 8, 2003.

This study was supported by the Paul Kayser International Award of Merit in Retina Research, Houston, Tex (Dr J. A. Shields), Macula Foundation, New York, NY (Dr C. L. Shields), the Noel T. and Sara L. Simmonds Endowment for Ophthalmic Pathology, Wills Eye Hospital (Dr Eagle), Philadelphia, Pa, and the Eye Tumor Research Foundation, Philadelphia (Dr C. L. Shields).

Corresponding author: Carol L. Shields, MD, Oncology Service, Wills Eye Hospital, Thomas Jefferson University, 840 Walnut St, Philadelphia, PA 19107.

REFERENCES

7. Shields CL, Shields JA, De Potter P, Himmelstein BP, Meadows AT. The effect of


