Hyperfractionated External Beam Radiation Therapy in the Treatment of Murine Transgenic Retinoblastoma

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Objective: To determine the in vivo efficacy of hyperfractionated external beam radiation therapy (EBRT) in comparison with standard daily EBRT in a murine model of heritable retinoblastoma.

Methods: Two hundred twenty eyes from 6-week-old simian virus-40 large T-antigen–positive mice were treated with a total dose of EBRT ranging from 10-76 Gy (1000 to 7600 rad). One hundred ten eyes underwent EBRT administered in 2.0-Gy (200-rad) fractions once per day. Forty-two eyes received hyperfractionated EBRT administered in 1.2-Gy (120-rad) fractions twice per day, while 48 eyes received EBRT twice daily in fractions of 5.0 Gy (500 rad). Twenty eyes served as untreated controls. All eyes were obtained for histopathologic examination and graded positive if any tumor was present.

Results: A dose-dependent inhibition of ocular tumor was observed for EBRT in these transgenic retinoblastoma mice. The tumor control dose for 50% of eyes (TCD50) treated with 2.0 Gy fractions of EBRT was 45 Gy (4500 rad) when treatments were administered once daily. A significant increase in tumor control was observed when treatments were administered twice per day at fractions of 1.2 Gy, resulting in a TCD50 of 33 Gy (3300 rad) (P = .003). A further increase in tumor control was observed when twice-daily EBRT was administered in 5.0 Gy fractions resulting in a TCD50 of 28 Gy (2800 rad).

Conclusions: Hyperfractionated EBRT safely and effectively controls intraocular retinoblastoma in this transgenic animal model. Use of hyperfractionation allows for a reduction in total radiation delivered dose, while shortening the total treatment time.

Clinical Relevance: This treatment approach may be applicable in the management of pediatric retinoblastoma by maintaining excellent tumor control, while reducing treatment-associated complications.

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Retinoblastoma, arising from embryonic photoreceptor cells in the eye, is the most common primary intraocular malignancy in childhood.1,2 Children with a germline RB gene mutation are predisposed to bilateral eye involvement and a lifetime predisposition for cancer involvement.3,5 Recent evidence suggests an increase in the incidence of retinoblastoma during the last 40 years, possibly because of more successful methods of treatment resulting in improved patient survival and/or increased mutation rates.6 Current treatment methods include enucleation, scleral plaque irradiation, cryotherapy, photocoagulation, external beam radiotherapy (EBRT), chemotherapy, and combined therapeutic modalities.2,7,8 Retinoblastoma is a highly radiosensitive tumor and can be effectively treated with ionizing EBRT.9-11 In the past, EBRT was administered most commonly to children with bilateral retinoblastoma that was untreatable with local therapies.12 This treatment modality was generally preferred if a tumor was larger than 15 mm in diameter, if it was located adjacent to the optic disc or fovea, if multiple tumors were present, or if extensive vitreous seeding of tumor cells was noted.9 Failures of EBRT leading to enucleation were often due to progression of vitreous seeds, recurrences from previously existing tumors, or the development of new intraocular tumors.13 Although EBRT has been shown to improve ocular and visual prognosis in retinoblastoma survivors, it is associated with multiple complications.14-18 Current concerns related to the application of radiotherapy in the treatment of retinoblastoma have focused on delayed complications including radiation-enhanced second tumor...
MATERIALS AND METHODS

The study protocol was approved by the University of Miami School of Medicine Animal Care and Use Review Board, Miami, Fla. All experiments in this study were conducted in accordance with the Association for Research in Vision and Ophthalmology guidelines for the use of animals in ophthalmologic and vision research.

Two hundred twenty simian virus-40 large T-antigen transgene-bearing mice were treated, as described below, beginning at 6 weeks of age. Transgenic animals were identified through polymerase chain reaction analysis of tail DNA. Transgene-positive animals develop bilateral, heritable retinoblastoma that resembles human retinoblastoma. All animals were anesthetized with a combination of intraperitoneal ketamine hydrochloride and xylazine hydrochloride and topical proparacaine hydrochloride for the purpose of tail blood extraction. No anesthesia was required for EBRT.

ORBITAL EBRT

Two hundred twenty mouse eyes were treated, as described herein, with fractionated EBRT with the use of a 10-mV x-ray machine (Clinac-2100; Varian Medical Systems, Inc, Palo Alto, Calif). Animals were briefly immobilized for treatment in specially constructed cages and shielded to minimize radiation to the midline structures (Figure 1). Animals were placed, head first, in the immobilization tube, treatment ports were confirmed, and radiation was delivered at 3.24 Gy/min (324 rad/min) to a field size of 7.0 x 7.0 cm, with the heads of 4 mice radiated simultaneously. This treatment effectively focuses therapy to the orbit, while shielding nonocular midline structures. The total treatment doses ranged from 24-76 Gy (2400 to 7600 rad).

One hundred ten eyes underwent EBRT administered in 2.0-Gy fractions delivered once per day, 5 days per week. Forty-two eyes received EBRT twice per day, 5 days per week, in fractions of 1.2 Gy, while 48 eyes received similar treatment in fractions of 5.0 Gy. All radiation treatments delivered twice per day were administered 6 to 8 hours apart. An additional 20 eyes from simian virus-40 T-antigen–positive transgenic mice served as untreated controls.

HISTOPATHOLOGIC STUDY OF TRANSGENIC MICE

At 16 weeks of age, all animals were killed with an overdose of ketamine and xylazine. Both eyes were enucleated and immediately immersion-fixed in 10% formalin. The eyes were sectioned serially with 80-µm sections stained with hematoxylin-eosin. Light microscopic examination was performed on all histopathologic sections in a masked fashion. The eyes were graded positive for tumor development if any histopathologic evidence of tumor was present. Eyes were also evaluated for evidence of corneal, lenticular, retinal, or scleral toxic effects.

STATISTICAL ANALYSIS

To determine the dose-response relationships among the different EBRT fractionation schemes, the data from all experimental groups were combined and subjected to probit statistical analysis. Total radiation dose was entered as a linear predictor in a maximum likelihood probit regression model. The model was analyzed for goodness-of-fit, and logistic regression analysis was used to estimate the relationship between EBRT dose and tumor control.

RESULTS

All untreated control eyes exhibited large intraocular tumors (Figure 2). None of the eyes treated with a total radiation dose below 10 Gy (1000 rad) exhibited tumor control (Table 1).

A dose-dependent inhibition of ocular tumor was observed for EBRT in these transgenic retinoblastoma mice at all treatment fractions and dosing strategies. Increas-
ing the frequency of radiation treatment, while decreasing the fraction delivered per treatment, was significantly associated with increased tumor control when 1.2 Gy hyperfractionation was compared with 2.0 Gy conventional daily fractionation (Figure 3 and Figure 4). The tumor control dose for 50% of the eyes (TCD50) treated once daily with 2.0 Gy fractions was 45.0 Gy (4500 rad) at 16 weeks of age. The tumor control dose decreased to 33.9 Gy (3390 rad) for animals treated with radiation in 1.2-Gy fractions twice per day. Increasing the fractionated dose from 1.2-Gy to 5.0-Gy fractions twice per day further increased tumor control, resulting in a TCD50 of 28.0 Gy (2800 rad). However, corneal and lenticular damage was observed in all of the eyes treated with these high-dose fractions of 5.0 Gy (Figure 5). None of the animals treated with dose fractions of either 2.0 or 1.2 Gy showed evidence of toxicity.

Probit regression analysis was used for estimating the relationship between dose and tumor control, and logistic regression analysis demonstrated that the dose-response curves were significantly different among the 3 treatment groups (P = .003) (Figure 6).

### COMMENT

External beam radiation therapy has been used for the treatment of retinoblastoma for more than 50 years. Focal delivery to the eye, new megavoltage machines, and lens-sparing techniques have contributed to the increasing efficacy of this treatment modality, improving ocular and visual prognoses, while decreasing treatment-related morbidities.34 The current study demonstrates that hyperfractionation (increasing the frequency of radiation treatment, while decreasing the fraction delivered per treatment) is significantly associated with increased tumor control in a murine transgenic model of retinoblastoma.

A wide range of EBRT dose fractionation schedules have been used previously in the management of childhood retinoblastoma; however, the choice has been largely empirical.13,33,35 Treatment variables have recently become more defined, with the most common fractionation schemes using fractions ranging from 1.8 Gy (180 rad) to 2.0 Gy administered once per day with total doses not exceeding 50.0 Gy. Radiation fractions greater than 2.0 Gy per treatment are potentially associated with severe ocular and pericocular morbidity, particularly in eyes receiving concomitant chemotherapy. Total radiation doses exceeding 45.0 Gy may lead to lacrimal gland damage and decreased tear production. At total doses exceeding 50.0 Gy, radiation retinopathy may be seen.37

Despite the recent development of more defined treatment schedules, optimal dose-fractionation schedules are still unclear. The use of this unique mouse model of retinoblastoma has allowed for preclinical modeling of a variety of treatment regimens. As reported previously, the transgenic mouse model used in this study has histopathologic, immunocytochemical, electron microscopic, and clinical characteristics similar to those of human retinoblastoma.38-41 In addition, the course of tumor development parallels that of human retinoblastoma.

Hyperfractionated radiotherapy refers to the delivery of an increased number of fractional radiation doses of smaller than conventional size in an overall treatment time comparable to, or shorter than, that required for conventional EBRT.22 Hyperfractionation is usually accomplished by delivering more than 1 fraction per day in sessions ranging from 4 to 8 hours apart. The rationale underlying the application of hyperfractionation is to exploit the differential radiosensitivities of the target volume tissues showing acute effects from those showing late effects, to decrease the rate of complications, while improving local tumor control.22 Radiosensitivity is related inversely to the degree of differentiation of the tissue and directly related to mitotic and mitochondrial activity.37 Although retinoblastoma has been characterized as a moderately well-differentiated tumor, it may be particularly susceptible to hyperfractionated EBRT because of the tumor’s high mitotic activity.

A phase 1/2 study was conducted to investigate the efficacy and toxicity of hyperfractionated radiotherapy in 136 children with poor-prognosis brainstem tumors.30 Children with brainstem tumors were treated with hyperfractionated EBRT at 1.1 Gy (110 rad) twice daily (minimum of 4-6 hours between fractions) to 66.0 Gy (6600 rad) in 6 weeks. Most patients (71% [24/34]) improved clinically during the course of treatment. This prompted a dose escalation from 66.0 Gy to 70.2 Gy (7020 rad) and then to 75.6 Gy (7560 rad).31 The results of that study investigating escalated doses of hyperfractionated radiotherapy demonstrated a trend toward increased overall survival and disease-free survival at 70.2 Gy. Although neurologic improvement at 70.2 Gy was similar to that at 66.0 Gy (77% [30/39]), survival times differed; the probability of survival at 2 years was only about 5% for children treated at 66.0 and 75.6 Gy and was 22% for children treated at 70.2 Gy.

Similarly, 284 children with rhabdomyosarcoma were treated with hyperfractionated EBRT to 59.4 Gy (5940 rad) at 1.1 Gy per fraction, twice daily with a 6- to 8-hour interfraction interval, to assess the toxicity of hyperfractionated radiation combined with chemothera-
plitude.32 This was a 10% calculated dose increase of the biologically effective dose over conventional daily fractionation. No deaths or unusual toxic effects were reported in any of the patients treated (0% [0/284]).

Thirty-nine patients with Ewing sarcoma were treated by several different regimens incorporating hyperfractionated EBRT at 1.2 Gy per fraction, twice daily with a 6-hour interfraction interval.33 Total dose delivered depended on response to chemotherapy and was 50.4 Gy (5040 rad) for complete regression, 55.2 Gy (5520 rad) for 50% resolution, and 60 Gy (6000 rad) for 50% resolution of soft tissue mass. The 5-year local control rate for the 3 different systemic regimens and the hyperfractionated radiosurgery as given in this paragraph was similar to that of other studies, indicating a possible application in the treatment of large retinoblastomas.44-49 Clinical trials have also demonstrated the benefit of chemoreduction combined with adjuvant therapy.46,50-52 Although effective in reducing the tumor volume in small to medium-sized tumors, chemoreduction plus local cryotherapy, laser photocoagulation, thermotherapy, or plaque radiation therapy has not proved as effective in the treatment of large tumors (Reese-Ellsworth stage IV and V).9 Systemic administration of chemotherapy, as a primary treatment modality, has been used in small to medium-sized tumors to decrease the tumor volume of intraocular retinoblastoma.44-46 Clinical trials have also demonstrated the benefits of chemoreduction combined with adjuvant therapy.46,50-52 Although effective in reducing the tumor volume in small to medium-sized tumors, chemoreduction plus local cryotherapy, laser photocoagulation, thermotherapy, or plaque radiation therapy has not proved as effective in the treatment of large tumors (Reese-Ellsworth group 5).44-46 Recent studies indicate that EBRT is often needed as salvage therapy after failure of systemic chemotherapy in large retinoblastomas.44-46 In addition, new reports of patients treated with EBRT indicate lower incidences of second tumor development among long-term survivors of heritable retinoblastoma than previously reported.5,54 The current study further establishes a clear dose-effect relationship of EBRT delivered in hyperfractionation. These results suggest that lowering the fraction per radiation dose from the conventional 1.8 to 2.0 Gy once per day to a fractionated dose of 1.2 Gy twice per day may provide enhanced tumor control, while reducing the overall treatment dose.

In the past 10 years, systemic chemotherapy has largely replaced EBRT in the treatment of medium to large tumors (Reese-Ellsworth stage IV and V).9 Systemic administration of chemotherapy, as a primary treatment modality, has been used in small to medium-sized tumors to decrease the tumor volume of intraocular retinoblastoma.44-46 Clinical trials have also demonstrated the benefits of chemoreduction combined with adjuvant therapy.46,50-52 Although effective in reducing the tumor volume in small to medium-sized tumors, chemoreduction plus local cryotherapy, laser photocoagulation, thermotherapy, or plaque radiation therapy has not proved as effective in the treatment of large tumors (Reese-Ellsworth group 5).44-46 Recent studies indicate that EBRT is often needed as salvage therapy after failure of systemic chemotherapy in large retinoblastomas.44-46 In addition, new reports of patients treated with EBRT indicate lower incidences of second tumor development among long-term survivors of heritable retinoblastoma than previously reported.5,54

### Table: Tumor Response for Each Subgroup of Conventional Single-Daily and Hyperfractionation Twice-Daily Therapy at Each Dose Fraction

<table>
<thead>
<tr>
<th>Fraction, Gy (rad)</th>
<th>Schedule</th>
<th>Total Dose, Gy (rad)</th>
<th>No. of Eyes</th>
<th>No. (%) With Complete Tumor Control</th>
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<tbody>
<tr>
<td>1.20 (120)</td>
<td>BID</td>
<td>24.0 (2400)</td>
<td>10</td>
<td>1 (10)</td>
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<tr>
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<td>8</td>
<td>6 (75)</td>
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<tr>
<td>1.20 (120)</td>
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<td>10</td>
<td>9 (90)</td>
</tr>
<tr>
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<td>QD</td>
<td>24.0 (2400)</td>
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<td>0</td>
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<td>19</td>
<td>10 (53)</td>
</tr>
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<td>12</td>
<td>9 (75)</td>
</tr>
<tr>
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<td>8 (80)</td>
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<td>10</td>
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<td>10</td>
<td>8 (80)</td>
</tr>
<tr>
<td>5.0 (500)</td>
<td>BID</td>
<td>50.0 (5000)</td>
<td>8</td>
<td>8 (100)</td>
</tr>
</tbody>
</table>

*BID indicates twice daily; QD, daily.
nal beam radiation therapy remains a valuable treatment option, particularly in the management of tumors with extensive vitreous seeding, for tumors located near the optic nerve, or after the failure of systemic chemotherapy.

Hyperfractionated radiotherapy may prove most useful in combination with adjuvant therapies, or as salvage therapy for patients who have previously undergone systemic chemotherapy. To decrease the risk of secondary malignancy after chemotherapy treatment, new studies have investigated the local delivery of chemotherapy via subconjunctival injection, peribulbar injection, episcleral balloon delivery, and coulomb-controlled iontophoresis. The use of hyperfractionated EBRT in combination with local chemotherapy may provide effective treatment, while minimizing toxicity and the increased incidence of secondary malignancy.

This study demonstrates improved efficacy of twice-vs once-daily radiotherapy without increased toxicity in...
short-term follow-up within a transgenic murine model of heritable retinoblastoma. Limitations of this study are inherent in the application of animal modeling to human clinical disease. These data establish a framework for pilot clinical evaluation of hyperfractionation in the treatment of childhood retinoblastoma requiring EBRT. Hyperfractionated radiotherapy may offer the benefits of increased tumor control in patients with childhood retinoblastoma, while minimizing the total radiation dose and thus potentially lowering the risk of treatment-related complications.

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REFERENCES
