

Paclitaxel in the Treatment of Retinal Tumors of LH beta-Tag Murine Transgenic Model of Retinoblastoma

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PURPOSE. The purpose of this study was to investigate tumor control efficacy of paclitaxel in the treatment of retinal tumors harbored by the LH beta-Tag murine transgenic model of retinoblastoma.

METHODS. LH beta-Tag-positive mice ($n = 5$) 10 weeks of age received two 20- μ L subconjunctival injections delivered with a 72-hour interval to right eyes only. Paclitaxel was dissolved in 100% dimethyl sulfoxide (DMSO) and delivered at doses of 0.5 mg, 0.25 mg, 0.125 mg, 0.0625 mg, 0.0313 mg, and 0.0152 mg in a 20- μ L volume. Control mice received two subconjunctival injections of DMSO or of saline solution to right eyes only or they remained untreated. Eyes were analyzed at 16 weeks of age for residual tumor burden, which was measured by gauging the cross-sectional area of largest tumor focus.

RESULTS. Linear regression analysis of tumor burden demonstrates a statistically significant ($P < 0.001$) dose-response relationship between paclitaxel concentration and tumor size. Transient ocular toxicities were observed after treatment, but most had subsided at the time of analysis, 6 weeks after injection. After histologic assessment, the 0.25-mg paclitaxel dose had effectively reduced tumor burden and was associated with minimal toxicity, including mild conjunctival chemosis and fibrosis, corneal epitheliopathy, and corneal edema.

CONCLUSIONS. Subconjunctival delivery of paclitaxel effectively inhibits intraocular tumor burden in the LH beta-Tag model of retinoblastoma. This treatment modality may represent a novel strategy for the clinical management of retinoblastoma. (*Invest Ophthalmol Vis Sci.* 2007;48:3437-3440) DOI:10.1167/iovs.06-0796

Retinoblastoma is one of the most prevalent intraocular malignancies of childhood.¹ It arises from mutations in the retinoblastoma gene, the first tumor suppressor locus to be characterized.² Current treatments such as external beam radiotherapy and systemic chemotherapy, which consist of DNA damaging-agents such as carboplatin and etoposide, are associated with significant morbidity and potential mortality.³ This is particularly problematic with patients with *rb-1* germline mutations because of the potential for secondary tumors.⁴⁻⁶ Therefore, treatments that function through alternative mechanism are under investigation.^{7,8}

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Supported by National Institutes of Health Grants R01 EY01629 and Center Grant P30 EY014801 and by an unrestricted grant to the University of Miami from Research to Prevent Blindness, Inc.

Submitted for publication July 12, 2006; revised December 14, 2006, and February 20, 2007; accepted May 16, 2007.

Disclosure: F. Suárez, None; M.-E. Jockovich, None; E. Hernandez, None; W. Feuer, None; J.-M. Parel, None; T.G. Murray, None

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Paclitaxel is an antimitotic therapeutic agent that stabilizes microtubules and disrupts normal spindle formation during metaphase.⁹ It is as an effective treatment for a wide range of cancers¹⁰⁻¹³ but has been used primarily for breast, ovarian, lung, cervical, and endometrial cancer. In these primary cancers it has been commonly used as adjuvant therapy, and it has been routinely tested as monotherapy against cancer in animal models.^{14,15} To date it has been locally delivered only to the eye to treat experimental proliferative vitreoretinopathy¹⁶; only transient ocular toxicities have been reported.^{17,18}

Because of its poor water solubility, it is necessary to dissolve paclitaxel in a lipophilic solvent. The commonly used solvent, cremophor, has associated toxicities^{19,20}; therefore, we opted to use dimethyl sulfoxide (DMSO). Originally used as a commercial solvent, DMSO has the propensity for therapeutic use and the facility to act as a vehicle for drug delivery because of the ease with which it penetrates membranes. Paclitaxel itself has previously been diluted in 30% DMSO to treat New Zealand rabbits for experimental proliferative vitreoretinopathy; no retinal toxicities were associated with DMSO.¹⁶ Lenticular changes that have the appearance of lens opacity, however, have been repeatedly reported in rabbits, mice, and several other animals.²¹⁻²⁴ To date this unique lenticular change has not been reported in humans.²³

The purpose of the present study was to evaluate the efficacy of paclitaxel as monotherapy in reducing retinal tumor growth using the LH beta-Tag mouse model of retinoblastoma.

METHODS

The study protocol was approved by the School of Medicine Animal Care and Use Review Board of the University of Miami. All experiments were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmologic and Vision Research.

The LH beta-Tag transgenic mouse model used in this study has been characterized previously.²⁵⁻²⁷ Briefly, a highly expressed transgene drives retinal tumor development by overexpression of the SV40 large T antigen. In these transgenic animals, bilateral retinal tumors develop that resemble human retinoblastoma. When the animals are 10 weeks of age, tumors are typically moderate in size (occupying approximately 20%-25% of the retinal area). When the animals are 16 weeks of age, retinal tumors usually fill the orbit.

LH beta-Tag mice (six animals per treatment group) were treated at 10 weeks of age. This number was determined with a power study (Solo Power Analysis program; BMDP Statistical Software, Los Angeles, CA) based on pilot studies from our laboratory.

Subconjunctival Injections in Transgenic Mice

Only right eyes received subconjunctival injections of paclitaxel; left eyes remained untreated. In our treated controls only right eyes received either 100% DMSO or saline; left eyes remained untreated. It should be noted that a small dose of any agent delivered to the right eye may reach the untreated fellow eye and for this reason data from the untreated fellow eye was not reported.

LH beta-tag mice received two subconjunctival injections of paclitaxel (LC Laboratories, Woburn, MA) dissolved in 100% DMSO (Sigma-Aldrich, Inc., St. Louis, MO) to the right eyes at doses of 0.5, 0.25,

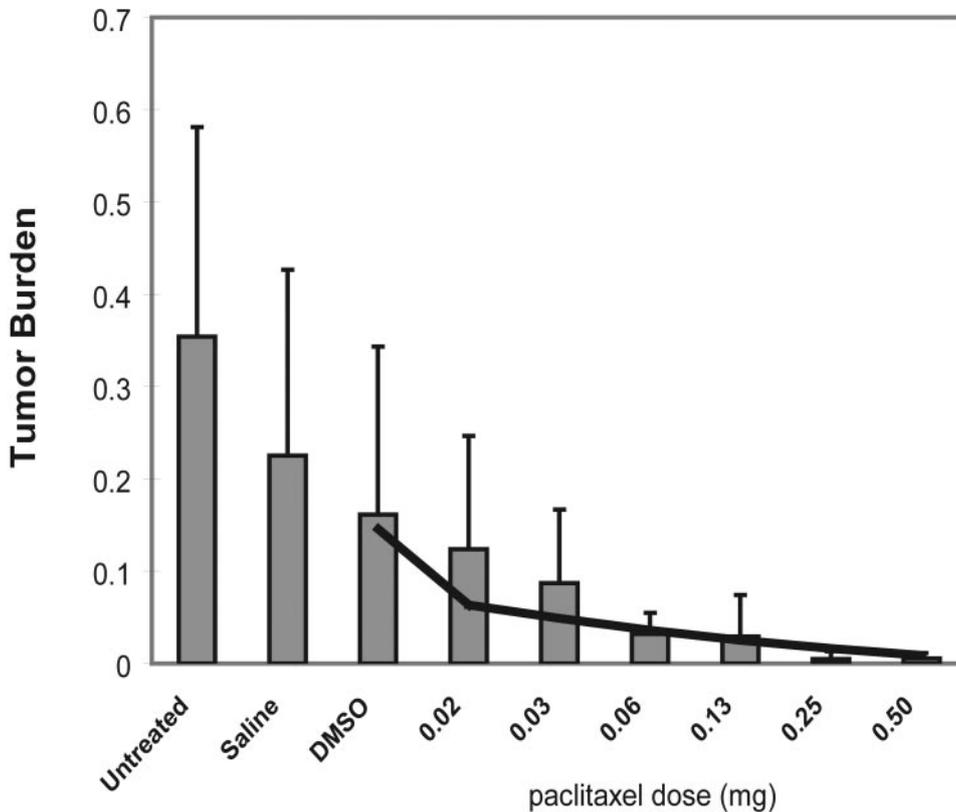


FIGURE 1. Dose-dependent response to paclitaxel. Tumor burden represents the ratio of the tumor area to the globe area of the cross-section with the largest tumor focus. Doses of paclitaxel represent milligrams in 20 μ L of 100% DMSO. Line displays the regression relationship of paclitaxel dose to tumor burden, which is linear with log dose. Linear regression analysis of paclitaxel response noted significant tumor burden reduction ($P < 0.001$). Note that tumor burden reductions for saline- and DMSO-treated control eyes were not statistically significant.

0.125, 0.0625, 0.0313, or 0.0152 mg in a 20- μ L volume. Injections were delivered 72 hours apart with a 33-gauge needle inserted into the superotemporal subconjunctival space.

Histopathologic Study of Transgenic Mice

At 16 weeks of age all animals, including those treated with different doses of paclitaxel, 100% DMSO only, or saline only and those left untreated, were humanely killed with CO₂ fumes. Both eyes of each mouse were enucleated and immediately immersion fixed in 10% formalin. The eyes were embedded in paraffin, sectioned serially in 5- μ m sections, and processed for standard hematoxylin-eosin (H&E) analysis. Light microscopic examination was performed on all histopathologic sections in a masked fashion. Microscopic images of all H&E stained sections (60 sections [5- μ m each] per eye) were obtained with a digital camera at a magnification of 40 \times . Tumor boundaries were traced, and areas were analyzed (Image Pro Express Software; Media Cybernetics, Silver Spring, MD) to determine the section with the largest tumor, which was then used in subsequent analyses.

Statistical Analyses

Tumor size response was fitted to the logarithm of paclitaxel dose with a linear regression model. Tumor/globe ratios were square root transformed to effect homogeneity of residual variance. We could have included three possible control groups in the analysis with a dose of zero: untreated, saline, or DMSO vehicle. To be conservative, we selected the DMSO vehicle that had the smallest mean tumor/globe ratio, even though it might not have been significantly different from that of the untreated or saline vehicle. A small positive constant, 10⁻³, was added to each dose because the log of zero was undefined. Statistical analysis was performed with SPSS (Chicago, IL) version 14.

RESULTS

Linear regression analysis of tumor burden demonstrates a statistically significant ($P < 0.001$) dose-response relationship

between paclitaxel concentration and tumor size (Fig. 1). Additional analysis (Dunnett test) compared the tumor response for each dose with the DMSO control. Doses of 0.125 and greater were significantly different than for control ($P = 0.037$, 0.004, and 0.005, respectively), whereas doses of 0.0625 and lower were not ($P = 0.146$, 0.812, 0.995; however, this could be attributed to artifact from sample size). Eyes that received two doses of 0.0156 mg paclitaxel had moderate tumor reduction, and those that received two doses of 0.25 mg had complete tumor reduction (Fig. 2). There also appears to have been a tumor response to DMSO and saline treatment, but statistical analysis suggests these treatments are not significantly different from untreated controls (Dunnett two-sided: $P > 0.05$, saline; $P > 0.05$, DMSO).

Ocular toxicities were evident in all treated eyes after therapy with paclitaxel and included conjunctival chemosis, corneal epitheliopathy, and corneal edema. Some paclitaxel-treated eyes displayed transient engorgement of iris vasculature. After two injections of high-dose (0.5 mg) paclitaxel, we observed severe toxicities in all eyes, including conjunctival fibrosis in all eyes and corneal perforation in two eyes. These eyes in the high-dose group remained severely affected at the time of enucleation, including the development of phthisis in one eye. During histologic assessment we also noted toxicity to the retina in one eye of the high-dose group.

Moderate toxicities, including conjunctival chemosis and fibrosis, corneal epitheliopathy, and corneal edema, were observed at the 0.25-mg and 0.125-mg doses. Corneal and conjunctival toxicities in eyes treated with the 0.25-mg and 0.125-mg doses appeared to ameliorate with time, becoming only mild at the time of enucleation. In eyes treated at doses lower than 0.125 mg, toxicities had completely resolved.

In addition to the toxicities mentioned, the eyes treated with paclitaxel also had lens opacity. However, unlike the other toxicities, lens opacity was also observed in eyes treated

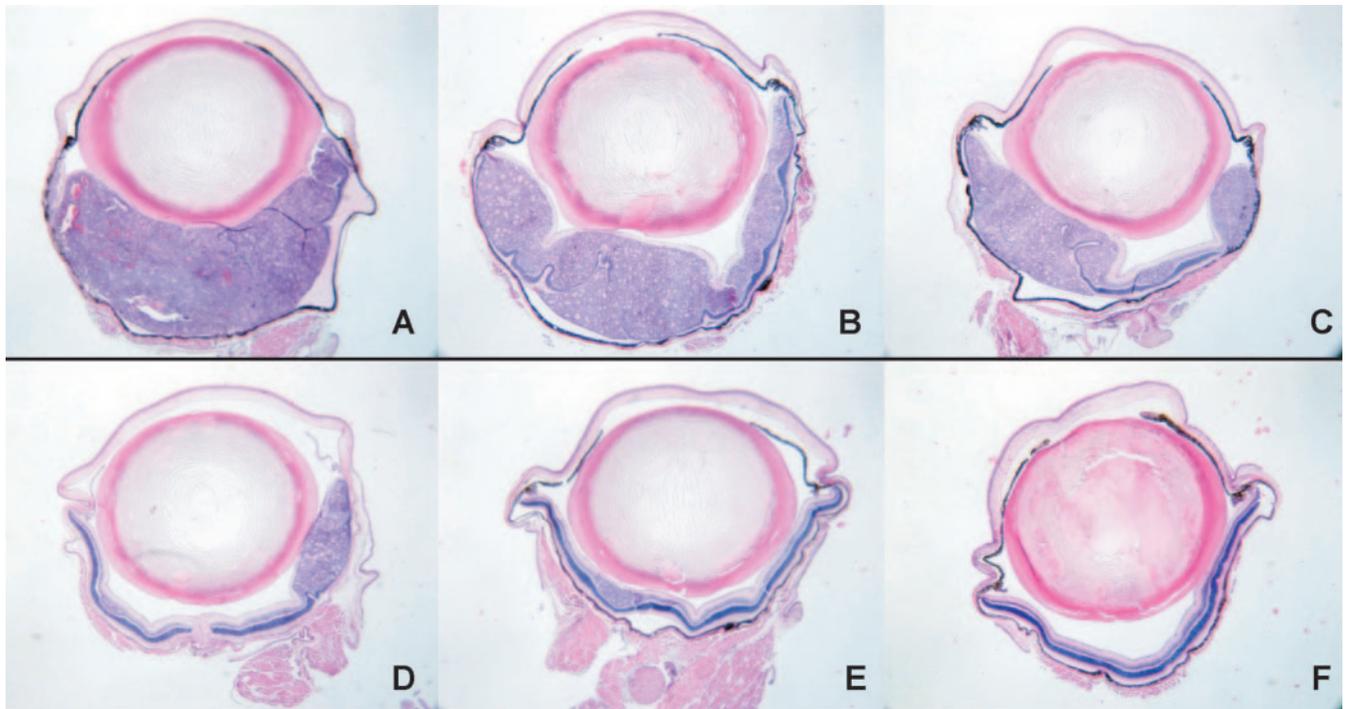


FIGURE 2. Histopathologic examination of enucleated globes of 16-week-old LH beta-Tag transgenic retinoblastoma mice. At 10 weeks of age, mice were left untreated (A) or were treated with saline (B), DMSO (C), 0.0156 mg paclitaxel (D), 0.0625 mg paclitaxel (E), or 0.25 mg paclitaxel (F). Note moderate tumor reduction for saline- and DMSO-treated control eyes (not statistically significant).

with DMSO alone and persisted without change in both treatments. Eyes treated with saline only and our untreated controls showed no signs of toxicity, as expected. No toxicities were observed in the untreated left eyes of mice that received two injections of paclitaxel, DMSO alone, or saline to their right eyes.

DISCUSSION

There are concerns about the morbidity and potential mortality associated with the standard chemotherapy regimen, which consists of three cycles of three drugs—carboplatin, etoposide, and vincristine—for retinoblastoma patients.^{28,29} The first two are mutagenic and have the potential to cause secondary tumors in patients with *rb-1* germline mutations.³ Vincristine is not mutagenic; however, secondary tumors have been reported in patients taking vincristine in a standard chemotherapy regimen.³⁰ Therefore, newer modalities, such as antiangiogenic treatments,^{7,8} are under investigation. In previous studies, paclitaxel has been used to treat experimental proliferative vitreoretinopathy¹⁶ and has also been used successfully to treat choroidal metastasis of breast cancer¹⁰; however, it has never been tested as a treatment for primary ocular malignancy.

Paclitaxel has been successful in the treatment of other cancers,^{10–15} and our data suggest that it can also be an effective agent for tumor reduction in patients with retinoblastoma. The fact that two small doses (e.g., 0.0152 mg) of paclitaxel delivered focally could significantly reduce tumor burden in our mouse model with little transient ocular toxicities is promising for children with retinoblastoma. However, paclitaxel delivered in combination with other therapeutic agents would result in greater tumor response and would reduce associated toxicities. We have shown, with our mouse model of retinoblastoma, that combination therapy is more effective than single therapy in the treatment of retinal tumors.^{7,31–33} Com-

bined therapy is used to treat various malignancies and is standard treatment for retinoblastoma patients. Future studies will primarily focus on delivering paclitaxel in combination with hyperthermia (laser therapy), antiangiogenic therapy, or another chemotherapy drug such as carboplatin.

Many studies have shown that taxanes have a beneficial synergistic effect with platinum compounds, such as carboplatin, against tumors.^{11,12} In one recent study, a combination of paclitaxel and carboplatin was used to successfully treat metastatic choriocarcinoma.¹⁰ In addition, results of a randomized phase III trial, published in 2003, demonstrated that paclitaxel in combination with carboplatin and etoposide is superior to the standard chemotherapy regimen (i.e., carboplatin, etoposide, and vincristine) in patients with small-cell lung cancer. Patients receiving a paclitaxel-containing regimen experienced significant long-term survival or progression-free survival and fewer drug-related toxicities than those receiving the standard regimen.¹¹

A previous difficulty with paclitaxel was the strong adverse effects associated with the delivery vehicle, the solvent cremophor.^{19,20} These toxicities included severe anaphylactoid hypersensitivity reactions, hyperlipidemia, abnormal lipoprotein patterns, aggregation of erythrocytes, and peripheral neuropathy. DMSO, on the other hand, has been well tolerated when used as a vehicle for the ocular delivery of drugs in mice, rabbits and humans. The only permanent adverse effect was lens opacity, evident in all eyes that received DMSO (study group and DMSO controls). Lens opacity has been reported in rabbits, mice, and several other animals, but no such effect has been reported in humans.²³ We thus believe that DMSO can be used to deliver paclitaxel to human eyes. DMSO appeared to decrease tumor burden in this mouse model, but it was not associated with a statistically significant reduction compared with untreated controls. Nonetheless, it is important to consider DMSO as a potentially active agent in the evaluation of

this animal model of retinoblastoma and when it is used as a delivery vehicle for other drugs.

In conclusion, focally delivered paclitaxel is effective in controlling tumor burden in our animal model and has the potential to be an effective treatment for patients with retinoblastoma. In human studies, however, it should be recommended as adjuvant therapy because complete tumor control was seen only at doses associated with transient ocular toxicities. Furthermore, adjuvant therapy has been the design of choice for cancer treatment with paclitaxel, as mentioned earlier.¹⁰⁻¹³ Monotherapy, however, should not be ruled out as an alternative because fibrin sealant, a biodegradable, semisolid medium for transscleral drug delivery, has been successful in delivering carboplatin as monotherapy.^{3,4} The compatibility of paclitaxel with fibrin sealant has yet to be investigated, but its success with carboplatin is promising.

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