Iontophoretic Delivery of Carboplatin in a Murine Model of Retinoblastoma

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Purpose. To evaluate the efficacy and dose–response of transcorneoscleral Coulomb controlled iontophoresis (CCI) of carboplatin in the treatment of retinal tumors of a murine model of retinoblastoma.

Methods. Thirty 6-week-old $LH_{BETA}T_{AG}$ mice underwent a total of six, serial iontophoretic treatments administered two times per week using a current density of 2.57 mA/cm^2 for 5 minutes. Fourteen animals received carboplatin treatments at concentrations of 1.4, 7.0, 10.0, or 14.0 mg/mL without current. Ten control mice underwent treatment with balanced saline solution.

Results. A dose-dependent inhibition of intraocular tumor was observed after repetitive iontophoretic treatment. At carboplatin concentrations of 7 mg/mL, 50% of the treated eyes (4/8) exhibited tumor control. No corneal toxicity was observed in eyes treated at carboplatin concentrations under 10 mg/mL.

Conclusions. CCI delivery of carboplatin safely and effectively controls intraocular tumors in a dose-dependent manner in this murine model of retinoblastoma. CCI is a noninvasive, painless option for the focal delivery of carboplatin. However, further clinical and laboratory research is needed before this method of drug delivery is available for children with retinoblastoma. (*Invest Ophthalmol Vis Sci.* 2006;47:3717–3721) DOI: 10.1167/iovs.06-0365

Retinoblastoma, the most common primary intraocular malignancy of childhood, comprises 3% of registered tumors in children under the age of 15 years.[1–3] Current treatments for retinoblastoma have led to a survival rate of greater than 95%.[4] However, current therapies are associated with long-term morbidity, particularly in children with germline *rb-1* mutations.[5,6] Thus, current therapies available for patients with retinoblastoma, particularly systemic chemotherapy and external beam radiation therapy, have recently come under increasing scrutiny.

External beam radiation, once used in the treatment of retinoblastoma, carries significant and potential limitations. First, delayed radiation complications including retinopathy, vasculopathy, optic neuropathy, cataract, and neovascular glaucoma occur.[7–10] Second, the heritable component of retinoblastoma appears to generate a cancer diathesis, with an increased incidence of second primary tumors in the field of radiation.[5,6,11]

Systemic chemotherapy, involving delivery of carboplatin alone or in combination with etoposide and vincristine, is currently standard in the management of bilateral retinoblastoma.[4,12–14] It is insufficient for tumor control and is often followed by adjuvant focal treatment such as cryoablation or laser therapy.[15–18] The treatment is associated with significant morbidity and potential mortality through drug-related toxicities and, similar to external beam radiation therapy, may have the potential to increase the risk of subsequent second cancers in childhood survivors with germ-line *rb-1* mutations.[19]

Focally delivered chemotherapy could carry the benefits associated with chemoreductive treatment and would conceivably spare patients the associated toxicity and mutagenic potential associated with the systemic delivery of chemotherapy. Focal carboplatin therapy delivered intravitreally or subconjunctivally has been shown to be effective in reducing the tumor burden in the $LH_{BETA}T_{AG}$ model of retinoblastoma.[20,21]

In this study, we describe the application of Coulomb controlled iontophoretic (CCI) delivery of carboplatin and evaluate its potential efficacy in the reduction of retinal tumor burden in the $LH_{BETA}T_{AG}$ model of retinoblastoma. The key to this study is the integration of CCI coupled with carboplatin chemotherapy, potentially providing a safer and more effective noninvasive treatment modality for transocular drug delivery.

Methods

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

The $LH_{BETA}T_{AG}$ mouse model has been described.[22,23] Briefly, a highly expressed transgene drives retinal tumor development by overexpression of the SV40 large T antigen. $LH_{BETA}T_{AG}$ mice develop bilateral retinoblastoma that resembles the human disease. Pathologic evidence of tumor is noted by 4 weeks of age, and tumors expand to fill the globe by 16 weeks of age. In this study, mice were treated at 6 weeks of age, when tumors are typically small, and residual tumor volume was analyzed at 16 weeks of age.

CCI Treatment Parameters

Optimal current density and charge application times were evaluated in 15 6-week-old $LH_{BETA}T_{AG}$. Animals were anesthetized with intramuscular injections of ketamine hydrochloride and xylazine. Treatments consisted of application of the positive custom CCI platinum electrode

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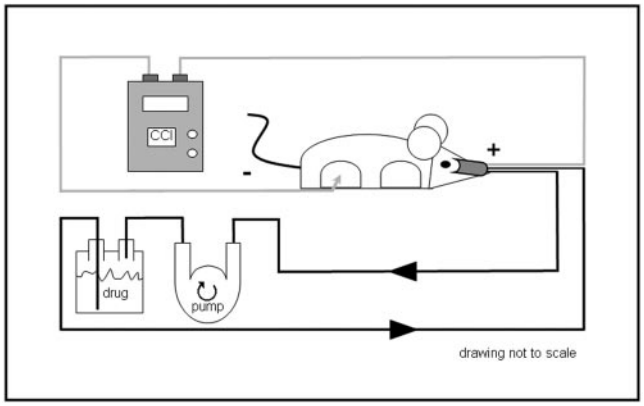

FIGURE 1. Schematic diagram of the CCI system, which includes a peristaltic pump to induce circulation and a constant drug flow. A transscleral applicator, containing the positively charged carboplatin solution, is the anode. The CCI electrode is placed on the sclera immediately posterior to the cornea. The negatively charged electrode is attached to 30-gauge needle inserted intramuscularly into the flank of the mouse during treatment, completing a closed circuit.

(active electrode) embedded in a polycarbonate material with silicon tubing to the mouse eye (Fig. 1). The CCI eyecup was placed on the eye, resting on the anterior sclera, abutting the corneal limbus (inner diameter of the CCI probe 3.14 mm, average diameter of the mouse cornea 3.0 mm). The negatively charged electrode (passive electrode) was attached to 30-gauge needle inserted intramuscularly into the flank of the mouse during treatment, completing a closed circuit. Carboplatin was dissolved in balanced saline solution to achieve the desired concentrations and resulted in a positively charged solution with pH of 5.8. Carboplatin solution was pumped continuously through the electrode at a rate of 0.55 mL/min and a suction of 5 mm Hg. Treatments were administered at current densities of 1.28, 2.57, 3.21, 3.85, and 5.14 mA/cm^2. Eyes were examined after treatment at 2, 5, and 10 minutes. After treatment at these different intensities, eyes were examined through a slit lamp for conjunctiva, cornea, and sclera damage. At the conclusion of the study treatments, all animals underwent serial ophthalmic examinations to evaluate for potential ocular toxicity.

Repetitive CCI in Transgenic Mice

Six-week-old, $LH_{BETA}T_{AG}$ mice underwent six serial CCI treatments delivered two times per week. All treatments were administered at a controlled current of 2.57 mA/cm^2, for 5 minutes. Thirty $LH_{BETA}T_{AG}$ mice were treated in the right eye with a total of six CCI treatments at concentrations of 1.4, 7, 10, or 14 mg/mL of carboplatin. Fourteen $LH_{BETA}T_{AG}$ mice were treated in the right eye with six sham CCI treatments (no current) at concentrations of 1.4, 7, 10, or 14 mg/mL of carboplatin. Ten control animals received six CCI treatments with balanced saline only, as a safety study for repetitive treatment. Fellow left eyes were always observed as the untreated control.

Histopathologic Study of Transgenic Mice

Animals were euthanatized at 16 weeks of age with an overdose of ketamine hydrochloride and xylazine hydrochloride. Both eyes were enucleated and immediately immersion-fixed in 10% formalin. Eyes were paraffin embedded, serially sectioned, and stained with hematoxylin-eosin. Sixty 5.0-μm axial sections were obtained beginning at a depth of 1.4 mm to ensure cross sectioning of the optic nerve in all eyes. Light microscopic examination was performed on all histopathologic sections in a masked fashion. Eyes were evaluated for evidence of corneal, lenticular, retinal, or scleral toxicity. Tumors were assessed by analyzing the histopathologic hematoxylin-eosin–stained eyes under the light microscope. Eyes were graded positive for tumor development if any histopathologic evidence of tumor was present and negative in the absence of tumor. Complete tumor control represented total absence of any tumor on gross and histopathologic review. Further, the presence of any tumor including microscopic tumor was considered a treatment failure in this study.

Statistical Analyses

Outcomes were analyzed, and a logistic regression program was used for statistical modeling to calculate dose–response curves (LogXact; Cytel Software, Cambridge, MA). Carboplatin concentration and tumor burden were analyzed as independent variables.

RESULTS

Safety of CCI

An investigation of CCI parameters for the treatment of retinoblastoma in this mouse model revealed an optimal transmittance of drug and absence of conjunctival, corneal, and scleral damage at current densities of 2.57, 3.21, or 3.85 mA/cm^2. No damage to the ocular tissues was observed in treatment times varying from 2 to 5 minutes in duration using this current density. A current density greater than 5.14 mA/cm^2 was associated with corneal–limbal toxicity at all treatment times evaluated. Signs of toxicity corresponded to a slight corneal epithelium damage and/or a very light superficial yellowing of the sclera reminiscent of a burn.

Iontophoretic Treatment of Transgenic Mice

All control eyes, including untreated fellow eyes, placebo-treated eyes, and eyes treated with carboplatin but no current, revealed large intraocular tumors (Fig. 2A). Experimental eyes treated at carboplatin concentrations of 1.4 mg/mL demonstrated a reduction in tumor volume when compared with untreated control eyes; however, none of the eyes at this dose

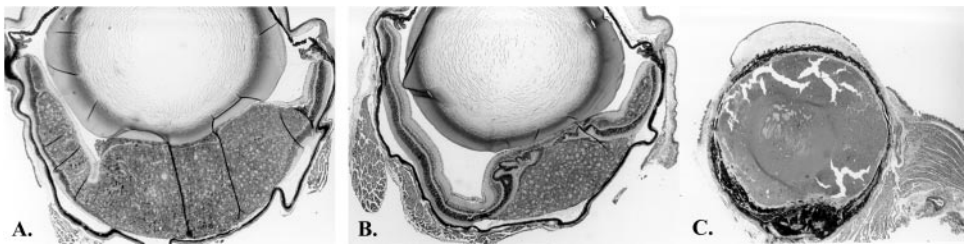

FIGURE 2. Histopathologic examination of enucleated globes of 16-week-old $LH_{BETA}T_{AG}$ mice after six serial CCI carboplatin treatments. (**A**) A control eye receiving balanced saline. A large retinal tumor is present. (**B**) Eye treated with 1.4 mg/mL of carboplatin. A moderate-sized tumor is present, demonstrating a reduction in tumor volume yet a lack of complete tumor control. (**C**) Eye treated with 14 mg/mL of carboplatin, showing an absence of tumor. Extreme carboplatin toxicity is evident at this concentration, resulting in phthisis. Hematoxylin and eosin; magnification ×7.

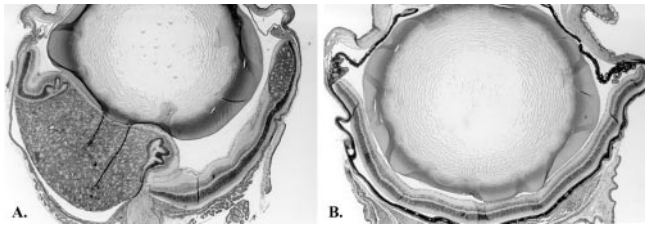

FIGURE 3. Histopathologic examination of enucleated globes of 16-week-old $LH_{BETA}T_{AG}$ mice after six serial CCI carboplatin treatments. (**A**) Eye treated with 7 mg/mL of carboplatin without applied current. Note large retinal tumor. (**B**) Eye treated with 7 mg/mL of carboplatin with applied current of 2.57 mA/cm^2. No evidence of cataract, scleral thinning, corneal decompensation or decomposition, or retinal abnormality is present. Note complete tumor control. Hematoxylin and eosin; magnification ×7.

exhibited complete tumor control (Fig. 2B). At carboplatin concentrations of 14 mg/mL (maximum solubility of carboplatin in BSS) all eyes (9/9, 100%) exhibited complete tumor control. Toxicity is evident in all animals treated at this concentration, with phthisis developing in some eyes (Fig. 2C). Histopathologic examination of enucleated globes of transgenic mice treated with six serial iontophoretic carboplatin applications at a concentration of 7.0 mg/mL, but without current, did not result in tumor control (Fig. 3A). Histopathologic examination of enucleated globes of transgenic mice treated with six serial iontophoretic carboplatin applications at a concentration of 7.0 mg/mL and a current of 2.57 mA/cm^2 for 5 minutes resulted in a complete tumor control in 50% of the treated eyes (4/8; Fig. 3B). There was no evidence of cataract, scleral thinning, corneal decompensation or decomposition, or retinal abnormality in any of the eyes treated at this dose. A dose-dependent inhibition of intraocular tumor was observed after repetitive CCI treatment (Fig. 4, Table 1). The tumor control dose for 50% of eyes treated (TCD_{50}) with iontophoretic carboplatin was determined by regression analysis to occur at a carboplatin concentration of 7.0 mg/mL. At carboplatin concentrations of 10 mg/mL most of the treated eyes revealed moderate corneal toxicity (epithelial keratopathy and/or corneal thinning) on histopathologic examination. All eyes treated with carboplatin concentrations of 14 mg/mL revealed complete corneal opacity as well as cataract. Retinal and choroidal thinning was also present. No ocular toxicity was observed in eyes treated at carboplatin concentrations less than 10 mg/mL.

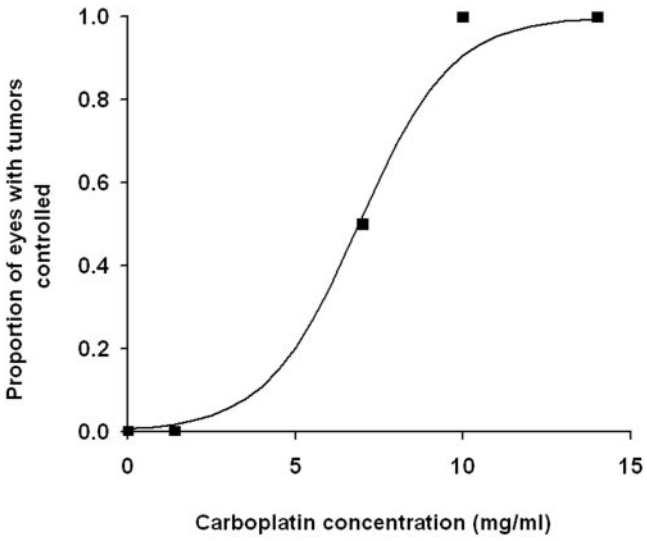

FIGURE 4. Tumor control dose–response curve of six serial CCI carboplatin deliveries. TCD_{50} = 7 mg/mL. Increased tumor control correlates directly with increased carboplatin concentration ($P < 0.001$).

TABLE 1. CCI Delivery of Carboplatin

Carboplatin Concentration (in the Eyecup)	Eyes with Corneal Toxicity *n*, (%)	Eyes with Complete Tumor Control *n*, (%)
14 mg/mL	9/9 (100)	9/9 (100)
10 mg/mL	5/6 (83)	6/6 (100)
7.0 mg/mL	0/8 (0)	4/8 (50)
1.4 mg/mL	0/7 (0)	0/7 (0)
BSS Control	0/7 (0)	0/7 (0)

LH_{BETA} T_{AG} mice underwent six serial CCI treatments delivered two times per week. Treatments were administered at a controlled current density of 2.57mA/cm^2, 5 minutes in duration.

Logistic regression analysis (LogXact; Cytel Software) was used to calculate a dose–response curve to estimate the relationship between carboplatin concentration and tumor control. The logistic regression analysis demonstrated that increased tumor control correlates directly with increased carboplatin concentration ($P < 0.001$).

DISCUSSION

Iontophoresis, the transfer of drugs across tissues through the application of a differential charge density, was first initiated in 1908 for the treatment of ocular disorders such as corneal ulcers, keratitis, and episcleritis.[24] In the past 10 years, numerous studies have documented the delivery of various drugs into ocular tissues in both the anterior and posterior chambers. Since then, other investigators have reported transcorneal and transscleral iontophoresis of antimitotic, antifungal, and antibacterial agents in the animal with varying degree of success.[25–32] Transscleral iontophoresis has been associated with damage to the retina and choroid, including thinning and disorganization of layers of the retina and occlusion of the choriocapillaris.[32] The delivery systems used by these researchers, however, did not address Coulomb's laws, resulting in retinal lesions and large variations in drug concentrations in the aqueous and vitreous. The Bascom Palmer Eye Institute, Ophthalmic Biophysics Center, has developed a CCI system specifically designed for ophthalmic application[30,33–36] This system is in clinical use in Europe[37] and in the United States.[38] The CCI used in this study maintains a constant current density (in milliamps per square centimeter), a constant concentration of carboplatin (in micrograms per milliliter), and a constant suction of 5 mmHg throughout the treatment period, resulting in effective transmission of drug into the ocular tissues without high charge density and fluctuation. This reduces complications such as tissue burning.

Iontophoresis uses a low-potential, continuous electrical field, incorporating two conductive electrodes to transfer ions across a tissue barrier. The electrode that contains the drug (the active electrode) is placed over the treatment site and a second electrode (the passive electrode), placed elsewhere on the body, closes the electrical circuit. The passive electrode has a large surface area, hence low impedance, thereby avoiding potential focal burns. The electrical field (E) and the potential (V) are selected to produce a small direct electrical current (DC) across the treatment tissue. Although the exact mechanism of action is unknown, it is postulated that the electrical field alters the epithelial interstitial spaces allowing the ionized drug to transfer across the treatment tissue.

When iontophoresis is used for drug delivery, the best candidate drug is a low-molecular-weight, charged molecule. According to electrical principals, if the molecule is positively charged, it is driven from the anode, and if it is negatively charged, it is driven from the cathode. Although iontophoresis delivery could be inversely dependent on the molecular weight of the candidate drug,[39] it has been shown that neutral molecules and high-molecular-weight molecules such as proteins can be delivered into the skin by the means of iontophoresis.[40] Of note, although the size of the molecule is an important factor influencing iontophoretic delivery, it has been demonstrated, using oligonucleotide penetration through the skin, that the base sequence and the base composition also affect steady state transport across the skin. Therefore, the molecular structure of the candidate drug may influence iontophoretic transport.

The chemical properties of carboplatin are uniquely attractive for application of CCI in the treatment of retinoblastoma. Carboplatin, an analogue of *cis*-platinum, is a crystalline powder with the molecular formula of $C_6H_{12}O_2PtO_4$ and a low molecular weight of 371.25 kDa. The polar molecule is neutral, but takes on a positive charge when dissolved in balanced salt solution. Carboplatin is soluble in balanced salt solution to a concentration of 14 mg/mL at a pH of 5.5. In vivo, carboplatin is metabolized to diaminoplatinum and a nonplatinum carboxyl moiety. Both carboplatin and its metabolite maintain the cytotoxic mechanism of action associated with the creation of inter- and intrastrand DNA linkages that impair cell replication.[41]

Previous studies in our laboratory have demonstrated the efficacy of focally delivered chemotherapy alone and in combination with other treatment modalities using subconjunctivally delivered carboplatin in a transgenic murine retinoblastoma model.[20,21,42,43] Another study has also shown clinically relevant carboplatin levels in the vitreous of primates after peribulbar and epibulbar administration.[44] Based on these favorable outcomes Abramson et al.[45] studied the efficacy and safety of subconjunctival administration of carboplatin in patients with retinoblastoma. After a median of three subconjunctival injections per eye, they found the treatment to be efficacious for retinoblastoma and suggested further evaluation of local delivery systems for carboplatin.

A major concern of focal chemotherapy delivered by subconjunctival injection is the risk of globe penetration during drug delivery. Invasion of the globe may result in tumor seeding along the needle track.[46] CCI offers a noninvasive method of drug delivery that is optimal for small charged molecules such as carboplatin. A study performed in our laboratory has demonstrated that transcorneoscleral CCI effectively delivers carboplatin and its metabolites into the retina, choroid, vitreous, and optic nerve in the rabbit eye.[47] This study showed that CCI not only delivers carboplatin to ocular tissues, but remarkable retinal tumor control can be attained at doses at which toxicity is not detected.

Corneal damage or toxicity after CCI treatment is a concern and may discourage the use of this technology in children with retinoblastoma. However, it is possible that the toxicity seen after CCI delivery to the mouse eye is due mainly to the anatomy of the mouse eye. The mouse cornea, unlike the human cornea, encompasses nearly half of the eye, almost to the equator. Therefore, it was difficult to avoid contact of the electrode with the cornea, which may have resulted in cornea epithelial damage. This may not be indicative of what is expected after treatment of a human eye. CCI delivery of carboplatin to the rabbit eye, which is more anatomically similar to the human eye, did not result in toxicity. Histopathology showed no signs of toxicity after six transscleral CCI applications (14 mg/mL carboplatin, 2.5 mA/cm^2, 20-minute duration/application).[47] Also, there were no reports of corneal toxicity in the CCI human studies.[33,37–38]

Transcorneoscleral iontophoretic delivery of carboplatin is a promising option for the focal delivery of carboplatin. CCI represents an effective and relatively noninvasive application of focal therapy for the treatment retinoblastoma. Dose-dependent inhibition of intraocular retinoblastoma was documented with an excellent therapeutic window and a tumor control dose for 50% of treated eyes occurring at 7 mg/mL for a treatment application time of 5 minutes. This study establishes a framework for further investigation into the clinical application of CCI carboplatin delivery in children with early and late-stage intraocular retinoblastoma.

References

1. Miller RW. Fifty-two forms of childhood cancer: United States mortality experience, 1960–1966. *J Pediatr.* 1969;75:685–689.
2. Devesa SS. The incidence of retinoblastoma. *Am J Ophthalmol.* 1975;80:263–265.
3. Tamboli A, Podgor MJ, Horm JW. The incidence of retinoblastoma in the United States: 1974 through 1985. *Arch Ophthalmol.* 1990; 108:128–132.
4. Shields CL, Shields JA. Diagnosis and management of retinoblastoma. *Cancer Control.* 2004;11:317–327.
5. Abramson DH, Frank CM. Second nonocular tumors in survivors of bilateral retinoblastoma: a possible age effect on radiation-related risk. *Ophthalmology.* 1998;105:573–579; discussion 579–580.
6. Abramson DH, Melson MR, Dunkel IJ, Frank CM. Third (fourth and fifth) nonocular tumors in survivors of retinoblastoma. *Ophthalmology.* 2001;108:1868–1876.
7. Schipper J, Imhoff SM, Tan KE. Precision megavoltage external beam radiation therapy for retinoblastoma. *Front Radiat Ther Oncol.* 1997;30:65–80.
8. Monge OR, Flage T, Hatlevoll R, Vermund H. Sightsaving therapy in retinoblastoma: experience with external megavoltage radiotherapy. *Acta Ophthalmol* (*Copenh).* 1986;64:414–420.
9. Shields JA, Shields CL, Sivalingam V. Decreasing frequency of enucleation in patients with retinoblastoma. *Am J Ophthalmol.* 1989;108:185–188.
10. Abramson DH, Ellsworth RM, Tretter P, Adams K, Kitchin FD. Simultaneous bilateral radiation for advanced bilateral retinoblastoma. *Arch Ophthalmol.* 1981;99:1763–1766.
11. Abramson DH, Beaverson KL, Chang ST, Dunkel IJ, McCormick B. Outcome following initial external beam radiotherapy in patients with Reese-Ellsworth group Vb retinoblastoma. *Arch Ophthalmol.* 2004;122:1316–1323.
12. Advani SH, Rao SR, Iyer RS, Pai SK, Kurkure PA, Nair CN. Pilot study of sequential combination chemotherapy in advanced and recurrent retinoblastoma. *Med Pediatr Oncol.* 1994;22:125–128.
13. Doz F, Khelfaoui F, Mosseri V, et al. The role of chemotherapy in orbital involvement of retinoblastoma: the experience of a single institution with 33 patients. *Cancer.* 1994;74:722–732.
14. Goble RR, McKenzie J, Kingston JE, Plowman PN, Hungerford JL. Orbital recurrence of retinoblastoma successfully treated by combined therapy. *Br J Ophthalmol.* 1990;74:97–98.
15. Beck MN, Balmer A, Dessing C, Pica A, Munier F. First-line chemotherapy with local treatment can prevent external-beam irradiation and enucleation in low-stage intraocular retinoblastoma. *J Clin Oncol.* 2000;18:2881–2887.
16. Brichard B, De Bruycker JJ, De Potter P, Neven B, Vermylen C, Cornu G. Combined chemotherapy and local treatment in the management of intraocular retinoblastoma. *Med Pediatr Oncol.* 2002;38:411–415.
17. Friedman DL, Himelstein B, Shields CL, et al. Chemoreduction and local ophthalmic therapy for intraocular retinoblastoma. *J Clin Oncol.* 2000;18:12–17.
18. Wilson MW, Rodriguez-Galindo C, Haik BG, Moshfeghi DM, Merchant TE, Pratt CB. Multiagent chemotherapy as neoadjuvant treatment for multifocal intraocular retinoblastoma. *Ophthalmology.* 2001;108:2106–2114; discussion 2114–2115.

19. Benz MS, Scott IU, Murray TG, Kramer D, Toledano S. Complications of systemic chemotherapy as treatment of retinoblastoma. *Arch Ophthalmol.* 2000;118:577–578.
20. Harbour JW, Murray TG, Hamasaki D, et al. Local carboplatin therapy in transgenic murine retinoblastoma. *Invest Ophthalmol Vis Sci.* 1996;37:1892–1898.
21. Hayden BH, Murray TG, Scott IU, et al. Subconjunctival carboplatin in retinoblastoma: impact of tumor burden and dose schedule. *Arch Ophthalmol.* 2000;118:1549–1554.
22. Windle JJ, Albert DM, O'Brien JM, et al. Retinoblastoma in transgenic mice. *Nature.* 1990;343:665–669.
23. O'Brien JM, Marcus DM, Bernards R, et al. A transgenic mouse model for trilateral retinoblastoma. *Arch Ophthalmol.* 1990;108: 1145–1151.
24. Sarraf D, Lee DA. The role of iontophoresis in ocular drug delivery. *J Ocul Pharmacol.* 1994;10:69–81.
25. Hill JM, Kwon BS, Shimomura Y, Colborn GL, Yaghmai F, Gangarosa LP. Herpes simplex virus recovery in neural tissues after ocular HSV shedding induced by epinephrine iontophoresis to the rabbit cornea. *Invest Ophthalmol Vis Sci.* 1983;24:243–247.
26. Hill JM, Park NH, Gangarosa LP, et al. Iontophoresis of vidarabine monophosphate into rabbit eyes. *Invest Ophthalmol Vis Sci.* 1978; 17:473–476.
27. Kwon BS, Gangarosa LP, Burch KD, deBack J, Hill JM. Induction of ocular herpes simplex virus shedding by iontophoresis of epinephrine into rabbit cornea. *Invest Ophthalmol Vis Sci.* 1981;21:442–449.
28. Kwon BS, Gangarosa LP Sr, Green K, Hill JM. Kinetics of ocular herpes simplex virus shedding induced by epinephrine iontophoresis. *Invest Ophthalmol Vis Sci.* 1982;22:818–821.
29. Barza M, Peckman C, Baum J. Transscleral iontophoresis as an adjunctive treatment for experimental endophthalmitis. *Arch Ophthalmol.* 1987;105:1418–1420.
30. Behar-Cohen FF, Savoldelli M, Parel JM, et al. Reduction of corneal edema in endotoxin-induced uveitis after application of L-NAME as nitric oxide synthase inhibitor in rats by iontophoresis. *Invest Ophthalmol Vis Sci.* 1998;39:897–904.
31. Lam TT, Fu J, Chu R, Stojack K, Siew E, Tso MO. Intravitreal delivery of ganciclovir in rabbits by transscleral iontophoresis. *J Ocul Pharmacol.* 1994;10:571–575.
32. Lam TT, Fu J, Tso MO. A histopathologic study of retinal lesions inflicted by transscleral iontophoresis. *Graefes Arch Clin Exp Ophthalmol.* 1991;229:389–394.
33. Behar-Cohen F, El Aouni A, Le Rouic JF, Parel JM, Renard G, Chauvaud D. [Iontophoresis: past and future]. *J Fr Ophtalmol.* 2001;24:319–327.
34. Kralinger MT, Voigt M, Kieselbach GF, Hamasaki D, Hayden BC, Parel JM. Ocular delivery of acetylsalicylic acid by repetitive coulomb-controlled iontophoresis. *Ophthalmic Res.* 2003;35:102–110.
35. Voigt M, Kralinger M, Kieselbach G, et al. Ocular aspirin distribution: a comparison of intravenous, topical, and coulomb-controlled iontophoresis administration. *Invest Ophthalmol Vis Sci.* 2002;43:3299–3306.
36. Behar-Cohen FF, El Aouni A, Gautier S, et al. Transscleral Coulomb-controlled iontophoresis of methylprednisolone into the rabbit eye: influence of duration of treatment, current intensity and drug concentration on ocular tissue and fluid levels. *Exp Eye Res.* 2002;74:51–59.
37. Halhal M, Renard G, Courtois Y, BenEzra D, Behar-Cohen F. Iontophoresis: from the lab to the bed side. *Exp Eye Res.* 2004; 78:751–757.
38. Yoo SH, Dursun D, Dubovy S, et al. Lontophoresis for the treatment of paecilomyces keratitis. *Cornea.* 2002;21:131–132.
39. Turner NG, Ferry L, Price M, Cullander C, Guy RH. Iontophoresis of poly-L-lysines: the role of molecular weight? *Pharm Res.* 1997; 14:1322–1331.
40. Bhatia KS, Gao S, Freeman TP, Singh J. Effect of penetration enhancers and iontophoresis on the ultrastructure and cholecystokinin-8 permeability through porcine skin. *J Pharm Sci.* 1997; 86:1011–1015.
41. Duffull SB, Robinson BA. Clinical pharmacokinetics and dose optimisation of carboplatin. *Clin Pharmacokinet.* 1997;33:161–183.
42. Murray TG, Cicciarelli N, O'Brien JM, et al. Subconjunctival carboplatin therapy and cryotherapy in the treatment of transgenic murine retinoblastoma. *Arch Ophthalmol.* 1997;115:1286–1290.
43. Murray TG, Roth DB, O'Brien JM, et al. Local carboplatin and radiation therapy in the treatment of murine transgenic retinoblastoma. *Arch Ophthalmol.* 1996;114:1385–1389.
44. Mendelsohn ME, Abramson DH, Madden T, Tong W, Tran HT, Dunkel IJ. Intraocular concentrations of chemotherapeutic agents after systemic or local administration. *Arch Ophthalmol.* 1998; 116:1209–1212.
45. Abramson DH, Frank CM, Dunkel IJ. A phase I/II study of subconjunctival carboplatin for intraocular retinoblastoma. *Ophthalmology.* 1999;106:1947–1950.
46. Karcioglu ZA, Gordon RA, Karcioglu GL. Tumor seeding in ocular fine needle aspiration biopsy. *Ophthalmology.* 1985;92:1763–1767.
47. Hayden BC, Jockovich ME, Murray TG, et al. Pharmacokinetics of systemic versus focal Carboplatin chemotherapy in the rabbit eye: possible implication in the treatment of retinoblastoma. *Invest Ophthalmol Vis Sci.* 2004;45:3644–3549.

E R R A T U M

Erratum in: "Effect of CTCF-Binding Motif on Regulation of PAX6 Transcription" by Wu et al. (*Invest Ophthalmol Vis Sci.* 2006;47:2422–2429).

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