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Case Study

Severe Periocular Edema after Intraarterial Carboplatin Chemotherapy for Retinoblastoma in a Rabbit (*Oryctolagus cuniculus*) Model

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Endovascular microcatheter-based intraarterial (ophthalmic artery) chemotherapy is becoming widely used for the clinical treatment of intraocular retinoblastoma due to its apparent increased efficacy compared with traditional intravenous chemotherapy; however local ocular complications are not uncommon. Carboplatin is a chemotherapeutic agent used in both intravenous and intraarterial chemotherapy. We used rabbits to assess pharmacokinetics and ocular and systemic toxicity after intraarterial carboplatin infusion. Subsequent to unilateral intraarterial administration of carboplatin, severe unilateral or bilateral periocular edema occurred in 6 adult male New Zealand white rabbits. Time to onset varied from less than 4 h after administration (*n* = 3, 50 mg) to approximately 24 h afterward (*n* = 3, 25 mg). After becoming symptomatic, 5 of the 6 animals were promptly euthanized, and the remaining animal (25 mg treatment) was medically managed for 4 d before being euthanized due to intractable edema-related lagophthalmos. Globes and orbits from all 6 euthanized rabbits were harvested en bloc; whole-mount sections were prepared for histologic evaluation, which revealed drug-induced vasogenic edema in confined spaces as the main underlying pathogenesis. Transient and self-limiting periocular edema is a common side effect of intraarterial chemotherapy but is thought to occur predominantly with melphalan monotherapy or combination therapy using melphalan, carboplatin, and topotecan. The severity of this adverse consequence in rabbits was unexpected, and its use in the study was subsequently discontinued. Although the definitive cause for this vasotoxicity and striking clinical presentation is unknown, we suspect species-specific anatomic features and sensitivity might have contributed to amplified complications after intraarterial carboplatin chemotherapy of the eye. Due to the adverse effects of intraarterial carboplatin chemotherapy that we observed in 2 experimental cohorts of rabbits, we recommend caution regarding its use in this species.

Abbreviations: IAC, intraarterial chemotherapy; ICRB, International Classification of Retinoblastoma

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Retinoblastoma, the most common primary intraocular malignancy in infants and children, originates from the retina, frequently due to loss or mutation of the retinoblastoma gene, *RB1*, and may affect one or both eyes.[3] For the past 2 decades, the mainstay of globe-conserving therapy has been systemic intravenous chemotherapy[9,26] using a combination of agents, including vincristine, etoposide, and carboplatin, to achieve chemoreduction.[14] The International Classification of Retinoblastoma (ICRB)—a staging system that divides intraocular retinoblastomas into 5 stages (A through E)—aids in determining anticipated clinical responses to systemic chemotherapy and the likelihood the globe can be salvaged.[38] The use of primary systemic chemotherapy has led to a cure rate as high as 95% for localized, low to midgrade retinoblastoma (ICRB groups A through C).[3] Despite this success rate, systemic chemotherapy has particularly poor success eliminating subretinal and vitreous seeds— dissociated rafts containing tumor cells—seen with high-grade tumors (ICRB groups D and E), and management of these tumors often requires enucleation.[31] In addition, systemic chemotherapy is associated with acute toxicities, including cytopenia, gastrointestinal complications, and long-term potential for organ dysfunction.[9,26]

The recent introduction and widespread adoption of local routes of chemotherapy delivery, such as endovascular, microcatheter-based intraophthalmic artery chemotherapy has dramatically improved treatment success rates and globe salvage rates for eyes with more advanced disease (ICRB group D and even some group E eyes).[1,37] Although the mainstay of intraarterial chemotherapy (IAC) has been melphalan, additional chemotherapeutic agents traditionally used in intravenous treatment of retinoblastoma have been used via these local routes, either alone or in combination with melphalan, in an attempt to eliminate vitreous seeds.[9,12,22,25] IAC provides elevated intraocular drug concentrations, yielding greater success with globe salvage for more advanced intraocular retinoblastoma while minimizing systemic absorption and toxicity.[1,9,32,39,41] Regional chemotherapy delivered via arterial vasculature has been applied in many tumor types, including those of the brain,[5,27,44] bone,[6] head and neck,[15,42] and eye.[20,46,48]

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Although melphalan remains the backbone of most IAC treatments for retinoblastoma, melphalan-based regimens are associated with local ocular and vascular adverse events.[9,23] Recently, a small animal model of IAC has been developed in rabbits, thus permitting determination of toxicity and maximal tolerable dose for IAC treatments.[9] Currently rabbits are the only small animal model of IAC. Animals in this study contributed to assessing pharmacokinetics and ocular and systemic toxicity of chemotherapeutic agents to develop alternative approaches in the management of high-grade intraocular retinoblastoma.

Case Series

Young adult male New Zealand white rabbits (Charles River, Quebec, Canada) with an average weight of 3.0 ± 0.2 kg were singly housed in stainless steel and plastic caging (Allentown, Allentown, NJ) under controlled conditions (ambient room temperature, 61 to 72 °F; humidity, 30% to 70%) on a 12:12-h light:dark cycle. Rabbits were confirmed to be free of viral, bacterial, and parasitic diseases according to vendor testing. Rabbits received a commercial pelleted diet (no. 5326, Lab Diet, St Louis, MO) supplemented with hay and access to water without restriction. The environmental enrichment program included a combination of social, structural, sensory, and food items. All experimental procedures were approved by the Vanderbilt University IACUC and were conducted in an AAALAC-accredited facility in accordance with the *Guide for the Care and Use of Laboratory Animals*,[17] the Public Health Service Policy on Humane Care and Use of Laboratory Animals,[28] and the Animal Welfare Act.[4]

From June 2017 through December 2017, nontumor-bearing New Zealand white rabbits were used to determine response to intraarterial carboplatin chemotherapy, as detailed previously.[9] Briefly, the analgesic and anesthetic protocol consisted of preoperative ketoprofen (1 mg/kg SC) and buprenorphine (0.02 mg/kg SC) for analgesia, ketamine (45 mg/kg IM) and xylazine (9 mg/kg IM) for induction, and, after endotracheal intubation, anesthetic maintenance using isoflurane (maximum, 2.5%). Intraoperatively, animals received maintenance fluids (0.9% NaCl, 15 mL/kg/h IV). Endovascular access of the femoral artery was obtained via a femoral artery cut-down and a 4-French micropuncture system. After heparinization, a 1.5-French Marathon microcatheter was advanced over a Mirage microwire (Medtronic Neurovascular, Minneapolis, MN). Under fluoroscopic guidance, the dominant ophthalmic arterial blood supply to the right eye was assessed in each rabbit. The microcatheter was then navigated to the dominant artery, and arterial selection was confirmed angiographically by using 1 mL of intraarterial contrast agent. Carboplatin was delivered in a pulsatile fashion unilaterally to the right eye of each rabbit, via the right eye's dominant (internal or external) ophthalmic artery over a 5-min period, followed by a saline flush. When infusion was complete, the catheter was removed from the femoral artery, the artery ligated to ensure hemostasis, and the skin closed through a 2-layer closure. During recovery from anesthesia, animals were closely observed until sternal and then returned to their home enclosures. Ketoprofen (1mg/kg SC) was readministered once, at 24 h after the initial premedication dose.

Three rabbits were treated as a cohort with 50 mg carboplatin on the same day and developed severe, bilateral periocular edema in less than 4 h after treatment. All 3 rabbits were euthanized promptly by using intravenous sodium pentobarbital. In the next study (5 mo later), the dose was reduced to 25 mg in an effort to avoid or minimize any periocular swelling, and a second cohort of 4 rabbits was treated. No rabbits developed any periocular swelling within 4 h after intraarterial dosing of 25 mg carboplatin. Approximately 24 h after carboplatin treatment, we again observed periocular swelling, this time in 3 animals, albeit less severe and more delayed, thus triggering intensive clinical management. Two of the rabbits were noted to have periocular edema (one in the right eye only, one in both eyes) on the morning after intraarterial administration of 25 mg carboplatin to the right eye and were given buprenorphine (0.02 mg/kg SC), and antibiotic ophthalmic ointment was applied topically on the exposed tissue. Due to clinical progression, both were euthanized shortly after the initiation of treatment. The third rabbit had less severe periocular edema in the right eye only on the morning after intraarterial administration of 25 mg carboplatin to the right eye and was given buprenorphine (0.02 mg/kg SC) every 12 h for 3 d, dexamethasone (4 mg tapering to 1 mg) subcutaneously every 24 h, and TobraDex (Alcon, Fort Worth, TX) or antibiotic eye ointment every 8 h. This rabbit was closely monitored and evaluated by the research and veterinary staff throughout the treatment period. Ultimately, euthanasia was elected due to the inability to resolve clinical signs. The remaining rabbit from the second cohort of 4 animals that was treated with 25 mg of intraarterial carboplatin died of causes unrelated to IAC approximately 72 h after its procedure.

To summarize our findings across the 2 cohorts: after unilateral intraarterial administration of carboplatin, a total of 6 adult male New Zealand white rabbits presented with periocular edema. On becoming symptomatic, 5 of the 6 animals were promptly euthanized, and the remaining rabbit (25-mg dose), which had less severe clinical signs, was medically managed for 4 d before being euthanized on day 5 after IAC in light of intractable edema-related lagophthalmos. Ocular pathology in all 6 euthanized rabbits (12 orbits) was evaluated at necropsy. Over 2 separate experimental cohorts, 10 of 12 (83%) eyes from animals treated unilaterally with carboplatin via an ophthalmic artery developed severe unilateral (n = 2) or bilateral (n = 4) periocular edema (Figure 1). Time to onset varied from less than 4 h after administration (n = 3; dose, 50 mg; bilateral lesions in all 3 rabbits) to approximately 24 h after administration (n = 3; dose, 25 mg; bilateral lesions in 1 of the 3 animals). Globes and orbits were harvested en bloc by using an oscillating saw and fixed for 72 h in 10% neutral buffered formalin (StatLab, McKinney, TX), thus permitting assessment of the globe with all periocular structures intact. Specimens were decalcified in Immunocal (StatLab) and sectioned parasagittally. Sections were prepared as whole-organ mounts on 2×3-in. glass slides, stained with hematoxylin and eosin, and evaluated by a board-certified veterinary pathologist.

Histopathology from the 3 rabbits treated with 50 mg carboplatin and euthanized less than 4 h after administration revealed marked edema of the bulbar and palpebral conjunctiva, extraocular muscles, and choroid. Fibrin exudation, congestion, and mild heterophilic infiltrates were present also. Extraocular muscles demonstrated acute, multifocal myodegeneration and myonecrosis. No evidence of vasculitis was present at this time point. Lesions were alike in treated (right) eyes and similar but less severe in the affected but untreated (left) eyes. In the 2 rabbits treated with 25 mg carboplatin and euthanized approximately 24 h after administration, findings were as noted in the 50-mg treatment group, with the addition of more temporally advanced lesions, including heterophilic choroiditis, multifocal endoarteritis, and coagulation necrosis in lobes of the Harderian gland (Figure 2). Lesions were alike in treated (right) eyes and similar but less severe in the affected but untreated (left) eyes. In the single rabbit treated with 25 mg carboplatin and

Figure 1. Gross post-mortem image of the right eye of a rabbit that developed severe, bilateral periocular edema approximately 24 h after intraarterial administration of 25 mg carboplatin in the dominant ophthalmic artery of this eye and was euthanized upon becoming symptomatic. In humans who develop periocular swelling after ophthalmic artery chemosurgery, the condition is transient, not considered to be painful, and generally allowed to resolve without treatment.

euthanized after 4 d of medical management (i.e., later than the other 2 within the cohort), lesions had progressed to include necrotizing arteritis, venous thrombosis, coagulation necrosis in lobes of the Harderian and lacrimal glands, and retinal detachment, degeneration, and necrosis (Figure 3). These lesions suggested that the underlying pathogenesis is drug-induced arteritis and vasogenic edema, with swelling of orbital structures perpetuating circulatory compromise and causing further ischemic damage.

Discussion

In this study, we report on severe ocular complications after endovascular intraophthlamic artery infusion of carboplatin. On the basis of reported adverse events in human patients, we anticipated mild to moderate and potentially self-limited complications after carboplatin administration through the ophthalmic artery; a retrospective study in humans examining adverse events and patient survival rates after ophthalmic artery chemosurgery revealed that melphalan and carboplatin are the most frequently administered chemotherapeutic agents in the management of ICRB group D tumors, with transient and often self-limited eyelid edema or localized skin erythema as the most commonly reported adverse events after treatment.[1] However, the severity of periocular edema in our rabbits was unexpected and resulted in discussions regarding humane endpoints and, ultimately, euthanasia. Periocular swelling in humans is not considered to be painful; the swelling is allowed to resolve over time, and sometimes systemic steroids are given. Generally, no pain medication is required for this clinical side effect in humans. In contrast to humans, in whom the inflammation causes the eyelids to swell closed, periocular edema in our rabbits caused the eyelids to swell and the eye to stay open (lagophthalmos), raising concern for exposure keratopathy. Most human patients who developed periocular edema following IAC had been treated with intraarterial melphalan or with a combination of melphalan, carboplatin, and topotecan, and the assumption was that melphalan was the primary driver of this complication.[1,39] Our findings in rabbits indicate that carboplatin alone can cause periocular swelling.

Carboplatin, a platinum-based chemotherapeutic agent, exerts antitumor effects by crosslinking DNA, thereby blocking transcription, and generally is considered less toxic with fewer adverse effects than related drugs, such as cisplatin.[18,44] Nevertheless, systemic carboplatin has been associated with significant drug-related toxicities including bone marrow suppression, nephrotoxicity, and ototoxicity.[14] Complications of subtenon or periocular carboplatin in humans include optic neuropathy, retinitis, maculopathy, and orbital fat necrosis and fibrosis, retinal vascular occlusions, vitreous hemorrhage, ptosis, and optic nerve swelling.[24,31,33,34] In recent years, refinements in the IAC technique have reinvigorated prospective investigation into the safety and efficacy of this approach.[47] Localized vascular toxicities have been seen previously with endovascular administration of carboplatin via other routes and for other indications. In a pig model investigating intracarotid infusion of carboplatin (50 mg) with the blood–brain barrier permeabilizer RMP7, no adverse clinical effects of carboplatin were observed, and cerebrovascular vasculitis and thrombosis was attributed to iatrogenically embolized cotton fiber fragments.[30] A human case of severe ocular and orbital toxicity after intracarotid injection of 306 mg carboplatin for recurrent glioblastoma was associated with ipsilateral chemosis, vasculitis, and bullous retinal detachment,[44] thus bearing a striking resemblance to the lesions in the rabbits of our study. Other cases of postchemotherapeutic vasculopathy have been documented in patients who received intravenous carboplatin as a single agent or as a component of combination therapy.[16,21,36]

Using saline controls, we demonstrated previously that the IAC method alone is not associated with adverse effects.[8,9] Pulsatile hand injection is the preferred method by neurointerventionalists for this endovascular technique in clinical use.[2,7,13,19,29] The ultimate target vessel of the infusion is the central retinal artery, which arises at the distal end of the ophthalmic artery. Both humans and rabbits have multiple small vessels that branch from the ophthalmic artery, supplying the orbit and other periocular tissues. From the field of rheology, we know that laminar flow, as would be found with continuous pump infusion, favors streaming of drug along the walls of the lumen of the vessel and thus increases the amount of drug carried into these side vessels. In contrast, turbulent flow, as occurs with rapid, pulsatile, hand injections, favors drug flow through the middle of the lumen, and thus drug preferentially enters the central retinal artery at the end of the ophthalmic artery. Similarly, human patients are treated through 5-min infusion in clinical practice, and we deemed it important to mimic the clinical situation as closely as possible in the rabbit model.

Despite unilateral drug administration, a subset of rabbits developed bilateral periocular edema. The pathophysiology in veterinary species compared with humans is an important consideration in this phenomenon. We suspect that species-specific ocular vascular anatomy contributed to the observed complications of ocular IAC in rabbits. Interophthalmic artery anastomosis can occur in rabbits, and we were able to visualize this communication during angiographic mapping of the vasculature prior to carboplatin infusion. Therefore, we do not think that the contralateral edema is related to systemic recirculation of drug but is rather due to local delivery to the contralateral eye via this anastomosis.[10,35] Yet previous pharmacokinetic studies indicate that only a minuscule fraction of the total drug dose reaches the contralateral eye during unilateral ocular IAC administration.[9] We therefore suggest that rabbits are particularly sensitive to vasotoxic effects of carboplatin, and we advise caution regarding the use of intraarterial carboplatin chemotherapy

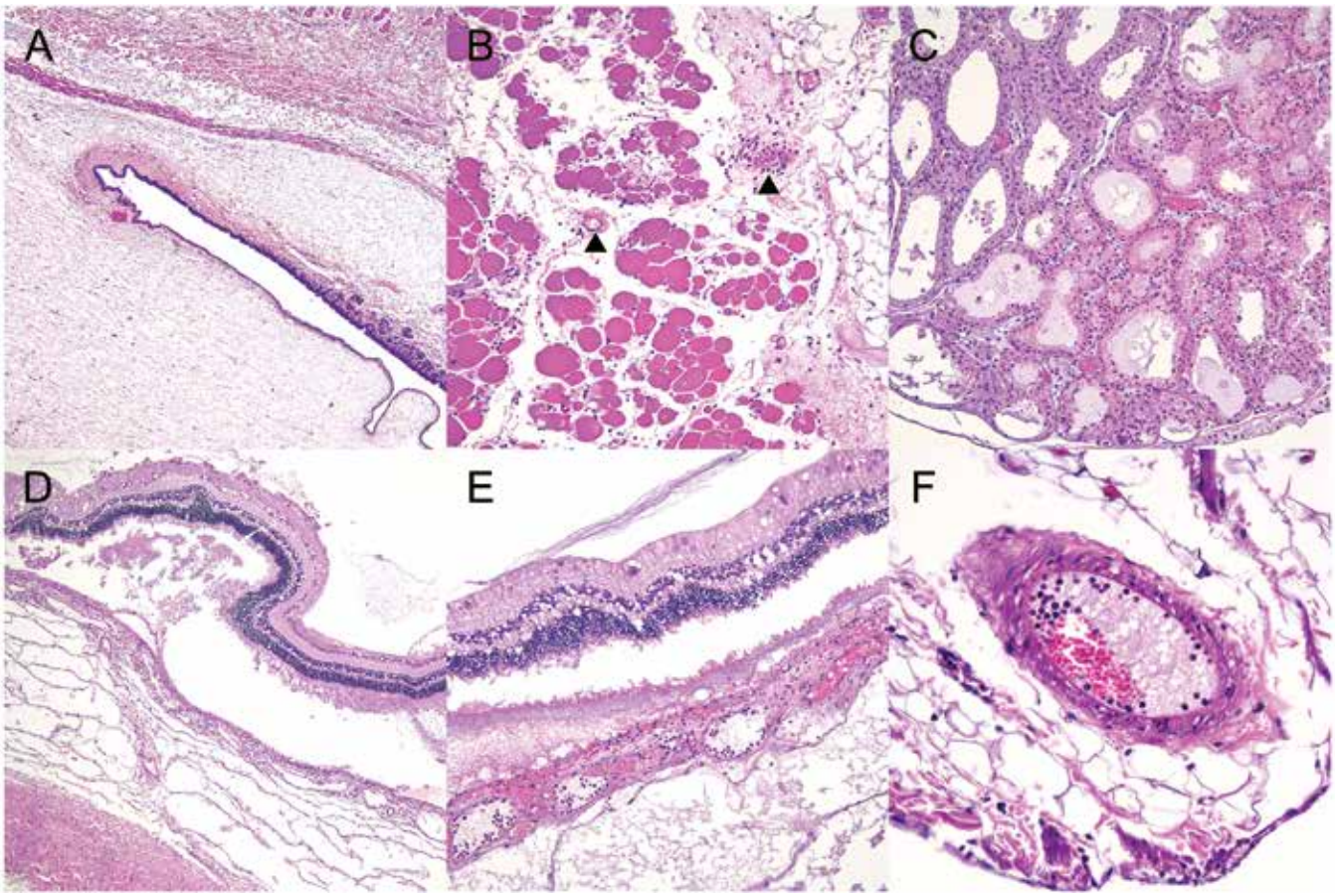

Figure 2. Histopathology of ocular tissues in a rabbit that received 25 mg intraarterial carboplatin. This rabbit was euthanized when it became symptomatic at 24 h postadministration. (A) Photomicrograph of the conjunctival fornix demonstrates severe edema of the bulbar and palpebral conjunctiva. Original magnification, 40×. (B) Inter- and intramuscular edema affecting extraocular muscles, as well as myodegeneration, myonecrosis, fibrin exudation, and heterophilic infiltrates. Two small-caliber arterioles demonstrate fibrin exudation and heterophilic exocytosis (arrowheads). Original magnification, 200×. (C) A focus of acute, focally extensive coagulation necrosis and heterophilic inflammation in the Harderian gland. Original magnification, 200×. (D) Marked choroidal edema and retinal separation at the level of the photoreceptor layer and retinal pigmented epithelium. Original magnification, 100×. (E) Higher magnification of the retina and choroid with choroidal edema, congestion, fibrin exudation, and acute heterophilic infiltrates. Original magnification, 200×. (F) A medium-caliber arteriole in the retroorbital space demonstrates endothelial loss, some pyknotic nuclear debris in the media, and heterophil margination. Original magnification, 400×.

in this species. Because the administration of carboplatin to the dominant ophthalmic artery was confirmed angiographically, it is unlikely the dual ophthalmic arterial supply contributed significantly to the pathogenesis. In addition, a 25-mg dose adjustment may represent an undercorrection relative to the blood supply to the rabbit eye. Compromised drainage of the retrobulbar venous plexus (e.g., due to periocular edema) could perpetuate exophthalmos.[45] Finally, although rabbits have a higher endogenous thrombin potential than humans, rats, sheep, and pigs,[40] suggesting that rabbits may be a generally prothrombotic model species, this feature is not thought to have played a significant role in our study.

The unexpected delayed appearance of clinical signs after a reduced (25 mg) carboplatin dose in our rabbits is interesting. A delayed-onset cerebrovascular accident has been reported in humans, with the proposed pathogenesis related to a lag time between endothelial injury and microthrombosis.[16] Although persistent periocular edema and erythema are successfully treated in humans after the administration of a tapered course of oral steroids,[24] subsequent treatment with subcutaneous dexamethasone and aggressive ocular lubrication in our single, medically managed rabbit was unsuccessful in alleviating symptoms, with lesions persisting despite treatment. Due to the severity of clinical signs after intraarterial carboplatin chemotherapy in 2 separate experimental cohorts of rabbits—first at the human dose of 50 mg and then at a dose that was adjusted to account for the size of the rabbit eye (25 mg)—the use of intraarterial carboplatin in the study was discontinued.

Histopathologic lesions in our cases strongly suggest that the primary pathogenesis is acute vasotoxic arteriolar damage with subsequent vasogenic edema. Potential relevant mechanisms include endothelial toxicity, vasculitis, perturbation of the clotting system, platelet activation, abnormality of thromboxane–prostacyclin homeostasis, autonomic dysfunction, and vasospasm.[36] The 50-mg treatment group likely lacked histologic evidence of vasculitis due to the brief time that elapsed between symptom development and euthanasia and the known lag between the initiation of clinicopathologic disturbances and detectable light microscopic lesions. Another mechanism might be the presence of preformed crossreactive antibodies that lead to immune complexes and trigger a hypersensitivity type reaction, as has been suggested for cutaneous urticarial eruptions and angioedema after oxaliplatin treatment in some human patients.[43]

Although the rabbit model of retinoblastoma has limitations, no other small animal models of IAC are available currently. Large animal species (for example, NHP and 70-kg Landrace pigs) do not match the size of the human babies who will ultimately benefit from retinoblastoma research, and retinoblastoma

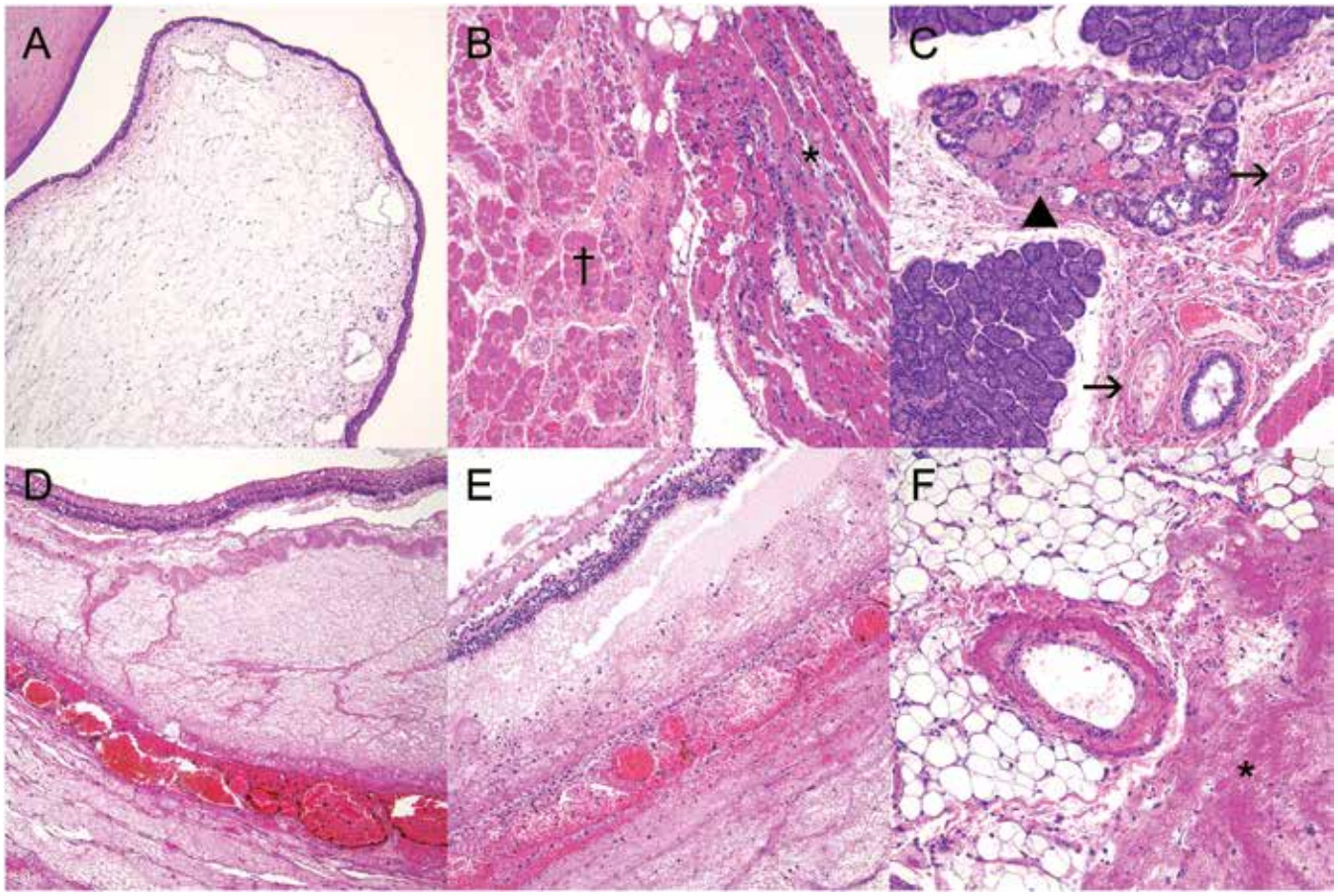

Figure 3. Histopathology of ocular tissues in a rabbit that received 25 mg intraarterial carboplatin and developed ocular edema 24 h after administration. This rabbit was medically managed for 4 d prior to euthanasia. Late findings included (A) persistence of severe edema in the conjunctiva. Original magnification, 100×. (B) Myodegeneration and myonecrosis (asterisk), coagulation necrosis (dagger), and heterophilic infiltration of the extraocular muscles. Original magnification, 200×. (C) Multifocal coagulation necrosis present in the lacrimal glands (arrowhead). Note that 2 arterioles in this field show fibrinoid vascular necrosis (arrows). Original magnification, 200×. (D) Severe subretinal and choroidal congestion, edema, and fibrin exudation. Original magnification, 100×. (E) Higher magnification of the retina and choroid shows degeneration and necrosis of the Purkinje cell, inner nuclear, plexiform, and rod and cone layers of the retina with marked choroidal fibrin exudation, hemorrhage, edema, and heterophilic inflammation. Original magnification, 200×. (F) A medium-caliber arteriole in the retroorbital space shows transmural necrosis (necrotizing arteritis). Note the large lake of fibrin exudation adjacent to the vessel (asterisk). Original magnification, 200×.

has not yet been modeled in these larger, higher order species. However, a xenograft model of retinoblastoma is available in rabbits,[9] and thus we can use this model to assess the efficacy and the toxicity of new drugs and techniques. Drugs given by intraarterial infusion in humans were first tested off-label in patients, rather than by using a preclinical model of the efficacy and safety of those drugs via this administration route. Therefore, the rabbit model of intraarterial chemotherapy is useful, despite being imperfect. Investigators using this model should exercise great caution if considering using carboplatin, even at a low dose, in rabbits. Veterinary care teams who may be working with a rabbit model of regional intraarterial chemotherapy should anticipate possible clinical complications and carefully consider their monitoring procedures, analgesic protocol, and humane endpoints.

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References

1. **Abramson DH, Daniels AB, Marr BP, Francis JH, Brodie SE, Dunkel IJ, Gobin YP.** 2016. Intra-arterial chemotherapy (ophthalmic artery chemosurgery) for group D retinoblastoma. PLoS One **11:**1–13. https://doi.org/10.1371/journal.pone.0146582.
2. **Abramson DH, Dunkel IJ, Brodie SE, Kim JW, Gobin YP.** 2008. A phase I/II study of direct intraarterial (ophthalmic artery) chemotherapy with melphalan for intraocular retinoblastoma initial results. Ophthalmology **115:**1398–1404e.1. https://doi.org/10.1016/j.ophtha.2007.12.014.
3. **Aerts I, Lumbroso-Le Rouic L, Gauthier-Villars M, Brisse H, Doz F, Desjardins L.** 2006. Retinoblastoma. Orphanet J Rare Dis **1:**1–11. https://doi.org/10.1186/1750-1172-1-31.
4. **Animal Welfare Act as Amended.** 2008. 7 USC §2131–2156
5. **Basso U, Lonardi S, Brandes AA.** 2002. Is intra-arterial chemotherapy useful in high-grade gliomas? Expert Rev Anticancer Ther **2:**507–519. https://doi.org/10.1586/14737140.2.5.507.

6. **Benjamin RS.** 1989. Regional chemotherapy for osteosarcoma. Semin Oncol **16:**323–327.
7. **Cloughesy TF, Gobin YP, Black KL, Viñuela F, Taft F, Kadkhoda B, Kabbinavar F.** 1997. Intra-arterial carboplatin chemotherapy for brain tumors: a dose escalation study based on cerebral blood flow. J Neurooncol **35:**121–131. https://doi.org/10.1023/A:1005856002264.
8. **Daniels AB, Froehler MT, Nunnally AH, Pierce JM, Bozic I, Stone CA, Santapuram PR, Tao YK, Boyd KL, Himmel LE, Chen SC, Du L, Friedman DL, Richmond A.** 2019. Rabbit model of intra-arterial chemotherapy toxicity demonstrates retinopathy and vasculopathy related to drug and dose, not procedure or approach. Invest Ophthalmol Vis Sci **60:**954–964. https://doi.org/10.1167/iovs.18-25346.
9. **Daniels AB, Froehler MT, Pierce JM, Nunnally AH, Calcutt MW, Bridges TM, LaNeve DC, Williams PE, Boyd KL, Reyzer ML, Lindsley CW, Friedman DL, Richmond A.** 2018. Pharmacokinetics, tissue localization, toxicity, and treatment efficacy in the first small animal (rabbit) model of intra-arterial chemotherapy for retinoblastoma. Invest Ophthalmol Vis Sci **59:**446–454. https://doi.org/10.1167/iovs.17-22302.
10. **de Oliveira IA.** [Internet]. 2012. Main models of experimental saccular aneurysm in animals. [Cited 28 March 2018]. Available at: http://www.intechopen.com/books/aneurysm https://doi.org/10.5772/50310
11. **Francis JH, Gobin YP, Brodie SE, Marr BP, Dunkel IJ, Abramson DH.** 2012. Experience of intra-arterial chemosurgery with single agent carboplatin for retinoblastoma. Br J Ophthalmol **96:**1270–1271. https://doi.org/10.1136/bjophthalmol-2012-301686.
12. **Francis JH, Gobin YP, Dunkel IJ, Marr BP, Brodie SE, Jonna G, Abramson DH.** 2013. Carboplatin $^{+}$/$^{-}$ topotecan ophthalmic artery chemosurgery for intraocular retinoblastoma. PLoS One **8:**1–7. https://doi.org/10.1371/journal.pone.0072441.
13. **Gobin YP, Cloughesy TF, Chow KL, Duckwiler GR, Sayre JW, Milanese K, Viñuela F.** 2001. Intraarterial chemotherapy for brain tumors by using a spatial dose fractionation algorithm and pulsatile delivery. Radiology **218:**724–732. https://doi.org/10.1148/radiology.218.3.r01mr41724.
14. **Hayden BC, Jockovich M-E, Murray TG, Voigt M, Milne P, Kralinger M, Feuer WJ, Hernandez E, Parel J-M.** 2004. Pharmacokinetics of systemic versus focal Carboplatin chemotherapy in the rabbit eye: possible implication in the treatment of retinoblastoma. Invest Ophthalmol Vis Sci **45:**3644–3649. https://doi.org/10.1167/iovs.04-0228.
15. **Homma A, Onimaru R, Matsuura K, Shinomiya H, Sakashita T, Shiga K, Tachibana H, Nakamura K, Mizusawa J, Kitahara H, Eba J, Fukuda H, Fujii M, Hayashi R.** 2017. Dose-finding and efficacy confirmation trial of the superselective intra-arterial infusion of cisplatin and concomitant radiotherapy for locally advanced maxillary sinus cancer (Japan Clinical Oncology Group 1212): Dose-finding phase. Head Neck **40:**475–484. https://doi.org/10.1002/hed.25001.
16. **Ikeda S, Sonoda K, Sumiyoshi M, Oki C.** 1999. Acute cerebrovascular toxicity after chemotherapy with carboplatin and cyclophosphamide for advanced ovarian carcinoma. Int J Clin Oncol **4:**52–53. https://doi.org/10.1007/s101470050025.
17. **Institute for Laboratory Animal Research**. 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
18. **Johnstone TC, Park GY, Lippard SJ.** 2014. Understanding and improving platinum anticancer drugs–phenanthriplatin. Anticancer Res **34:**471–476.
19. **Joshi S, Ellis JA, Ornstein E, Bruce JN.** 2015. Intraarterial drug delivery for glioblastoma mutiforme. J Neurooncol **124:**333–343. https://doi.org/10.1007/s11060-015-1846-6.
20. **Kondapavulur S, Cooke DL, Kao A, Amans MR, Alexander M, Darflinger R, Dowd CF, Higashida RT, Damato B, Halbach VV, Matthay KK, Hetts SW.** 2018. Estimation of intra-arterial chemotherapy distribution to the retina in pediatric retinoblastoma patients using quantitative digital subtraction angiography. Interv Neuroradiol **24:**214–219. https://doi.org/10.1177/1591019917749825.
21. **Lewis P, Waqar S, Yiannakis D, Raman V.** 2014. Unilateral optic disc papilloedema following administration of carboplatin chemotherapy for ovarian carcinoma. Case Rep Oncol **7:**29–32. https://doi.org/10.1159/000357912.
22. **Marr BP, Brodie SE, Dunkel IJ, Gobin YP, Abramson DH.** 2012. Three-drug intra-arterial chemotherapy using simultaneous carboplatin, topotecan and melphalan for intraocular retinoblastoma: preliminary results. Br J Ophthalmol **96:**1300–1303. https://doi.org/10.1136/bjophthalmol-2012-301925.
23. **Muen WJ, Kingston JE, Robertson F, Brew S, Sagoo MS, Reddy MA.** 2012. Efficacy and complications of super-selective intra-ophthalmic artery melphalan for the treatment of refractory retinoblastoma. Ophthalmology **119:**611–616. https://doi.org/10.1016/j.ophtha.2011.08.045.
24. **Mulvihill A, Budning A, Jay V, Vandenhoven C, Heon E, Gallie BL, Chan HSL.** 2003. Ocular motility changes after subtenon carboplatin chemotherapy for retinoblastoma. Arch Opthalmol **121:**1120–1124. https://doi.org/10.1001/archopht.121.8.1120.
25. **Munier FL, Gaillard MC, Balmer A, Soliman S, Podilsky G, Moulin AP, Beck-Popovic M.** 2012. Intravitreal chemotherapy for vitreous disease in retinoblastoma revisited: from prohibition to conditional indications. Br J Ophthalmol **96:**1078–1083. https://doi.org/10.1136/bjophthalmol-2011-301450.
26. **Murphree AL, Villablanca JG, Deegan WF 3rd, Sato JK, Malogolowkin M, Fisher A, Parker R, Reed E, Gomer CJ.** 1996. Chemotherapy plus local treatment in the management of intraocular retinoblastoma. Arch Opthalmol **114:**1348–1356. https://doi.org/10.1001/archopht.1996.01100140548005.
27. **Newton HB, Page MA, Junck L, Greenberg HS.** 1989. Intra-arterial cisplatin for the treatment of malignant gliomas. J Neurooncol **7:**39–45. https://doi.org/10.1007/BF00149377.
28. **Public Health Service.** [Internet]. 2015. Policy on humane care and use of laboratory animals. [Cited 18 December 2018]. Available at: https://grants.nih.gov/grants/olaw/references/phspolicylabanimals.pdf
29. **Requejo F, Marelli J, Ruiz Johnson A, Sampor C, Chantada G.** 2018. The technique of superselective ophthalmic artery chemotherapy for retinoblastoma: The Garrahan Hospital experience. Interv Neuroradiol **24:**93–99. https://doi.org/10.1177/1591019917738962.
30. **Riley MG, Kim NN, Watson VE, Gobin YP, LeBel CP, Black KL, Bartus RT.** 1998. Intra-arterial administration of carboplatin and the blood brain barrier permeabilizing agent, RMP-7: a toxicologic evaluation in swine. J Neurooncol **36:**167–178. https://doi.org/10.1023/A:1005751922174.
31. **Scelfo C, Francis JH, Khetan V, Jenkins T, Marr B, Abramson DH, Shields CL, Pe'er J, Munier F, Berry J, Harbour JW, Yarovoy A, Lucena E, Murray TG, Bhagia P, Paysse E, Tuncer S, Chantada GL, Moll AC, Ushakova T, Plager DA, Ziyovuddin I, Leal CA, Materin MA, Ji X-D, Cursino JW, Polania R, Kiratli H, All-Ericsson C, Kebudi R, Honavar SG, Vishnevskia-Dai V, Epelman S, Daniels AB, Ling JD, Traore F, Ramirez-Oritz MA.** 2017. An international survey of classification and treatment choices for group D retinoblastoma. Int J Ophthalmol **10:**961–967.
32. **Schaiquevich P, Buitrago E, Taich P, Torbidoni A, Ceciliano A, Fandino A, Asprea M, Requejo F, Abramson DH, Bramuglia GF, Chantada GL.** 2012. Pharmacokinetic analysis of melphalan after superselective ophthalmic artery infusion in preclinical models and retinoblastoma patients. Invest Ophthalmol Vis Sci **53:**4205–4212. https://doi.org/10.1167/iovs.12-9501.
33. **Schaiquevich P, Fabius AW, Francis JH, Chantada GL, Abramson DH**. 2017. Ocular pharmacology of chemotherapy for retinoblastoma. Retina **37:**1–10. https://doi.org/10.1097/IAE.0000000000001275 PubMed
34. **Schmack I, Hubbard GB, Kang SJ, Aaberg TM Jr, Grossniklaus HE.** 2006. Ischemic necrosis and atrophy of the optic nerve after periocular carboplatin injection for intraocular retinoblastoma. Am J Ophthalmol **142:**310–315. https://doi.org/10.1016/j.ajo.2006.02.044.
35. **Scremin OU, Sonnenschein RR, Rubinstein EH.** 1982. Cerebrovascular anatomy and blood flow measurements in the rabbit. J Cereb Blood Flow Metab **2:**55–66. https://doi.org/10.1038/jcbfm.1982.6.
36. **Serrano-Castro PJ, Guardado-Santervás P, Olivares-Romero J.** 2000. Ischemic stroke following cisplatin and 5-fluorouracil

therapy: a transcranial Doppler study. Eur Neurol **44:**63–64. https://doi.org/10.1159/000008197.

37. **Shields CL, Jorge R, Say EAT, Magrath G, Alset A, Caywood E, Leahey AM, Jabbour P, Shields JA.** 2016. Unilateral retinoblastoma managed with intravenous chemotherapy versus intra-arterial chemotherapy. Outcomes based on the international classification of retinoblastoma. Asia Pac J Ophthalmol (Phila) **5:**97–103. https://doi.org/10.1097/APO.0000000000000172.
38. **Shields CL, Mashayekhi A, Au AK, Czyz C, Leahey A, Meadows AT, Shields JA.** 2006. The International classification of retinoblastoma predicts chemoreduction success. Ophthalmology **113:**2276–2280. https://doi.org/10.1016/j.ophtha.2006.06.018.
39. **Shields CL, Shields JA.** 2010. Retinoblastoma management: advances in enucleation, intravenous chemoreduction, and intra-arterial chemotherapy. Curr Opin Ophthalmol **21:**203–212. https://doi.org/10.1097/ICU.0b013e328338676a.
40. **Siller-Matula JM, Plasenzotti R, Spiel A, Quehenberger P, Jilma B.** 2008. Interspecies differences in coagulation profile. Thromb Haemost **100:**397–404. https://doi.org/10.1160/TH08-02-0103.
41. **Taich P, Ceciliano A, Buitrago E, Sampor C, Fandino A, Villasante F, Lucena E, Romero L, Chantada GL, Schaiquevich P.** 2014. Clinical pharmacokinetics of intra-arterial melphalan and topotecan combination in patients with retinoblastoma. Ophthalmology **121:**889–897. https://doi.org/10.1016/j.ophtha.2013.10.045.
42. **Tsurumaru D, Kuroiwa T, Yabuuchi H, Hirata H, Higaki Y, Tomita K.** 2007. Efficacy of intra-arterial infusion chemotherapy for head and neck cancers using coaxial catheter technique: initial experience. Cardiovasc Intervent Radiol **30:**207–211. https://doi.org/10.1007/s00270-005-0272-0.
43. **Villée C, Tennstedt D, Marot L, Goossens A, Baeck M.** 2010. Delayed urticaria with oxaliplatin. Contact Dermat **63:**50–53. https://doi.org/10.1111/j.1600-0536.2010.01738.x.
44. **Watanabe W, Kuwabara R, Nakahara T, Hamasaki O, Sakamoto I, Okada K, Minamoto A, Mishima HK.** 2002. Severe ocular and orbital toxicity after intracarotid injection of carboplatin for recurrent glioblastomas. Graefes Arch Clin Exp Opthalmol **240:**1033–1035. https://doi.org/10.1007/s00417-002-0573-9.
45. **Williams D.** 2007. Rabbit and rodent ophthalmology. EJCAP **17:**242–252.
46. **Xue K, Liu A, Hui R, Zhang J, Qian J.** 2017. Retrobulbar ocular blood flow changes measured by colour Doppler imaging after intra-arterial chemotherapy in retinoblastoma. Br J Ophthalmol **101:**1419–1422. https://doi.org/10.1136/bjophthalmol-2016-310056.
47. **Yousef YA, Soliman SE, Astudillo PPP, Durairaj P, Dimaras H, Chan HSL, Héon E, Gallie BL, Shaikh F.** 2016. Intra-arterial chemotherapy for retinoblastoma: a systematic review. JAMA Ophthalmol **134:**584–591. https://doi.org/10.1001/jamaophthalmol.2016.0244.
48. **Yuan S, Friedman DL, Daniels AB.** 2017. Alternative chemotherapeutic agents for the treatment of retinoblastoma using the intra-arterial and intravitreal routes: a path forward toward drug discovery. Int Ophthalmol Clin **57:**129–141. https://doi.org/10.1097/IIO.0000000000000154.