Intraocular Concentrations of Chemotherapeutic Agents After Systemic or Local Administration

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Objectives: To investigate the concentrations of carboplatin and etoposide achieved in the aqueous and vitreous humors after intravenous infusion in nonhuman primates, and to investigate whether local administration of carboplatin might result in higher concentrations in the vitreous humor.

Methods: Macaca fascicularis primates were treated with 1 of 3 regimens: (1) intravenous carboplatin (18.7 mg/kg), etoposide (5 mg/kg), and vincristine sulfate (0.05 mg/kg), (2) peribulbar carboplatin (10 mg/mL), or (3) episcleral balloon carboplatin (10 mg/mL). Concentrations of chemotherapeutic agents were measured in the plasma and in the aqueous and vitreous humors.

Results: No measurable amount of etoposide was detected in the aqueous or vitreous humor after intravenous administration. Mean measured peak vitreous concentration of carboplatin after intravenous administration was 0.31 µg/mL, which was 1% of the peak plasma value. Mean measured peak vitreous concentrations of carboplatin after peribulbar or episcleral balloon administration were 2.38 µg/mL and 2.95 µg/mL, respectively, which represent 7.68- and 9.52-fold increases over the concentration achieved after intravenous administration. No serious toxic effect was observed in any animal.

Conclusions: Peribulbar and episcleral balloon administration of carboplatin seemed to be safe and resulted in higher vitreous concentrations than intravenous administration in this model. These results suggest that these alternate routes of delivery should be explored in children with vitreous seeding of retinoblastoma.

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Systemic chemotherapy is being used with increasing frequency to treat children with intraocular retinoblastoma in an attempt to avoid external beam radiation therapy and enucleation. External beam radiation therapy, although effective against retinoblastoma, is associated with increased mortality from radiation-induced tumors in patients carrying the RB-1 germ-line mutation. Chemotherapy has been hypothesized to be less likely than radiation therapy to induce second malignant neoplasms in these patients. Chemotherapeutic regimens vary, though most centers use combinations of vincristine, carboplatin, and an epipodophyllotoxin, either etoposide or teniposide. These regimens have been developed empirically, and it is not known which agents reach therapeutic concentrations in the eye, nor which are most effective against intraocular retinoblastoma. This knowledge would be particularly useful for designing a regimen to treat intravitreal retinoblastoma, which is often resistant to all other treatments, including radiation therapy.

We used a non–tumor-bearing primate model to determine the timing and degree to which 2 agents commonly used for intraocular retinoblastoma treatment (carboplatin and etoposide) penetrate into the humors of the eye. After intravenous delivery of chemotherapy, drug concentrations in aqueous and vitreous humors were measured over time. To determine whether local delivery could achieve concentrations comparable with those achieved by intravenous delivery of chemotherapy, intraocular concentrations of chemotherapeutic drugs achieved after intravenous administration were compared with concentrations obtained after peribulbar injection and episcleral balloon placement.

RESULTS

INTRAOCULAR CONCENTRATIONS

Measured peak plasma concentrations of etoposide after intravenous delivery occurred at either 30 or 60 minutes after delivery and gradually declined to approximately 50% of peak concentrations at 2
MATERIALS AND METHODS

CHEMOTHERAPY PROTOCOLS

Normal adult Macaca fascicularis monkeys were used. The animals were anesthetized with ketamine hydrochloride during the procedures. The research protocols were approved by the Memorial Sloan-Kettering Cancer Center Institutional Animal Care and Use Committee, New York, NY.

In the first group, 3 animals received intravenous vincristine sulfate, carboplatin, and etoposide in doses similar to those used in patients with retinoblastoma. Carboplatin (18.7 mg/kg) and etoposide (3 mg/kg) were administered simultaneously and infused over 1 hour, followed by an intravenous push of vincristine sulfate (0.05 mg/kg) at the end of the hour. Samples of aqueous and vitreous humors were drawn before any chemotherapy administration, then at half-hour intervals from completion of the chemotherapy infusion to 2 hours after the end of the infusion. Aqueous humor samples were drawn via clear corneal paracentesis from the superotemporal or superonasal area of the cornea. Vitreous humor samples were drawn transconjunctivally through the pars plana 3.5 to 4 mm posterior to the limbus in the superotemporal or superonasal quadrant. Venous blood and approximately 50 to 100 µL of aqueous and vitreous humors were sampled at each time point, alternating eyes.

In the second group, 3 animals received 1 mL of 10-mg/mL carboplatin injected transconjunctivally into the inferior peribulbar space of one eye. This concentration was chosen as the highest stable concentration recommended by the manufacturer. The injection was given far enough posteriorly to avoid any ballooning of the anterior conjunctiva and possible contamination of the pars plana vitreous sampling area, which was about 180° away. Samples of aqueous and vitreous humors were drawn every half hour from the ipsilateral eye, with samples for the zero time point taken before injection. At the final time point, blood was sampled as well.

In the third group, 2 animals had a recently described therapeutic episcleral balloon placed in the inferotemporal posterior peribulbar space of 1 eye, and 1 mL of a 10-mg/mL carboplatin solution was instilled into the catheter, delivering the carboplatin into the posterior peribulbar space. Aqueous and vitreous humor samples were taken from the ipsilateral eye every half hour for 2 hours, with time zero samples taken before instillation of the chemotherapy. At 2 hours, blood was sampled.

All animals had twice-weekly complete blood cell counts done for 3 weeks to observe for signs of bone marrow suppression. All animals had a postoperative ophthalmoscopic examination performed within the first several days after the procedure, with dilation and scleral depression to ensure that no peripheral retina trauma had occurred.

DRUG ASSAYS

Samples were assayed for etoposide by one of us (W.T.) with the use of high-performance liquid chromatography. The lower limit of detection of etoposide with this assay, remaining in linear range, is 0.2 µg/mL.10 Plasma samples were injected onto a 3-µm 6 × 40-mm column (Reliance ODS; Mac-Mod Analytical, Chadds Ford, Pa) at a flow rate of 1 mL/min. The mobile phase was 400 mL of methanol and 600 mL of 100-mmol/L sodium dihydrogen phosphate with 0.02 mmol/L diocetyl sodium sulfosuccinate and 0.3% triethylamine adjusted to pH 6 with phosphoric acid. The eluent was monitored at 235 nm at 0.002 absorbance units full scale.

Samples were analyzed for platinum by 2 of us (H.T.T. and T.M.) by flameless atomic absorption spectroscopy (SPECTRAA-300; Varian, Sugar Land, Tex) by use of a graphite tube atomizer as described by Madden et al11 and Mulder et al12 with minor modifications. Briefly, standards, controls, and samples were injected at a volume of 20 µL onto the graphite tube at a temperature of 25°C. The furnace temperature was increased from 25°C to 1300°C in 5 steps over 1.5 minutes, held at 1300°C (ashing) for 2 minutes, and raised to 2700°C over 60 seconds (atomization) and held for 5 seconds. Argon gas flow was 3 L/min. The hollow cathode lamp current was 10 mA; the spectral bandwidth was 0.7 nm at a monochromator wavelength of 265.9 nm, with a signal integration time of 5 seconds and background correction by hydrogen-2 lamp. A linear standard curve covering the range of 50 to 400 ng of platinum per milliliter was run at the start of each assay day and after each change to a new graphite tube. Aqueous and vitreous humor samples were diluted with 0.1N hydrochloric acid—0.25% Triton-X. Plasma ultrafilterate samples were diluted in 0.1N hydrochloric acid. All samples were diluted to achieve concentrations that were within the linear portion of the standard curve. Sample concentrations were determined by comparison of sample peak areas vs that of external standards by use of linear least-squares regression.

We were unable to assay vincristine because of technical difficulty of the assay method and the small sample volumes available.

hours. Mean measured peak plasma etoposide concentration was 18.8 µg/mL. No etoposide was detected in the aqueous or vitreous humor in any animal.

Measured peak plasma concentrations of carboplatin after intravenous administration occurred 30 minutes after completion of the infusion and gradually declined to approximately 50% of peak levels at 2 hours. Mean peak carboplatin concentration in plasma was 30.2 µg/mL. Carboplatin was detected in both aqueous and vitreous humors after intravenous delivery. Aqueous humor concentrations of carboplatin after intravenous delivery continued to rise over 2 hours in 2 animals and peaked at 60 minutes in the third animal. The mean measured peak aqueous humor concentration was 6.2 µg/mL, 20% of the peak serum value. Vitreous humor concentrations of carboplatin after intravenous delivery continued to rise over 2 hours in 2 animals and peaked at 90 minutes in the third animal (Table). The mean measured peak vitreous humor concentration was 0.31 µg/mL, 1% and 5%, respectively, of the peak plasma and aqueous humor concentrations.

PERIBULBAR INJECTION

Aqueous humor concentrations of carboplatin after peribulbar injection peaked at 90 or 120 minutes. The mean concentrations of carboplatin after intravenous delivery continued to rise over 2 hours in 2 animals and peaked at 90 minutes in the third animal (Table). The mean measured peak vitreous humor concentration was 0.31 µg/mL, 1% and 5%, respectively, of the peak plasma and aqueous humor concentrations.
The measured peak aqueous humor carboplatin concentration was 2.0 µg/mL, 32% of the peak aqueous humor value after intravenous carboplatin. At 2 hours, the mean concentration of carboplatin in the plasma was 0.89 µg/mL, 2.9% of the peak plasma concentration after intravenous administration.

Vitreous humor concentrations of carboplatin after peribulbar injection peaked at 30 minutes, dropped, then rose slowly until 120 minutes. The mean measured peak vitreous humor concentration was 2.38 µg/mL. This is more than 7 times (768%) the peak vitreous humor value after intravenous administration (Figure).

EPISCLERAL BALLOON

Aqueous humor concentrations of carboplatin after episcleral balloon delivery peaked at 60 minutes in both animals. Mean measured peak aqueous humor carboplatin concentration was 1.74 µg/mL, 28% of the peak aqueous humor value after intravenous delivery. At 2 hours the mean carboplatin concentration in the plasma was 0.056 µg/mL, 0.2% of the concentration after intravenous administration.

Vitreous humor concentrations of carboplatin after delivery through the episcleral balloon catheter peaked at 30 or 60 minutes, and the mean measured peak vitreous humor concentration was 2.95 µg/mL. This is more than 9 times (952%) the measured peak vitreous humor concentration after intravenous administration.

TOXIC EFFECTS

No animal showed ill effects in behavior or on dilated examination. One of the animals in the intravenous group had a mild transient neutropenia, which resolved without intervention.

There is little information regarding the concentrations of chemotherapeutic agents achieved in the aqueous and vitreous humors of the eye after intravenous administration to humans or animals. While intraocular retinoblastoma has been clearly demonstrated to be responsive to intravenous combination chemotherapy, the regimens used have been developed completely empirically. It is important to establish which effective agents reach therapeutic concentrations in the vitreous humor and to avoid those that do not and, therefore, may be contributing toxic effects without compensatory efficacy. Samples of aqueous and vitreous humors cannot be taken from eyes afflicted with retinoblastoma in vivo because of this cancer’s ability to metastasize outside the eye through needle biopsy sites. A primate model may be relevant to the clinical situation, but it must be noted that these results in a normal eye may not mirror those in an eye with retinoblastoma. The presence of tumors in the retina may disrupt the vascular anatomy sufficiently to increase concentrations of chemotherapeutic agents in the vitreous humor after intravenous administration. This study demonstrated that, after intravenous administration, 1% of the plasma carboplatin level was achieved in the vitreous humor. It should be noted, however, that the vitreous humor was not sampled beyond 2 hours, and it is possible that the true peak occurred later. This is somewhat less than the 20% to 30% of the plasma concentration that has been reported to be achieved in the cerebrospinal fluid, but the measured peak concentration in the vitreous humor of 0.31 µg/mL is comparable with the concentration of 0.19 µg/mL previously reported in a rabbit model. Clinical trials with single-agent intravenous carboplatin have demonstrated efficacy against both intraretinal and intravitreous retinoblastoma, and intravitreous and subcon-
junctional carboplatin have also been shown to inhibit growth of intraocular retinoblastoma in transgenic mice.\textsuperscript{17,18} Carboplatin is clearly an active agent against intraocular retinoblastoma, and there is a good rationale for its inclusion in multidrug regimens. In contrast, no etoposide was detected in the normal primate eye after intravenous delivery. This is similar to data indicating that less than 5% of the plasma etoposide concentration is achieved in the cerebrospinal fluid.\textsuperscript{14} This may indicate that etoposide is a poor choice for inclusion in multidrug regimens for the treatment of intravitreal retinoblastoma in children. Alternatively, as noted above, retinoblastoma-containing eyes may have alterations of barriers to drug delivery present in the non–tumor-bearing primate model. No phase II study of the effectiveness of etoposide in patients with intraocular retinoblastoma has been published.

Intravenous chemotherapy carries with it the attendant risks of systemic side effects. When only intraocular disease is being treated, a local administration system would be preferable. We compared carboplatin concentrations obtained after intravenous administration with concentrations achieved after peribulbar injection and episcleral balloon placement. The concentrations in the vitreous humor after local administration were 7 to 9 times higher than those obtained after intravenous administration. Local administration may be advantageous for treatment of vitreous seeds; however, intraretinal tumors may still require intravenous administration.

The concentrations in the aqueous humor after local administration were approximately 30% of those after intravenous administration, and the concentrations in plasma after local administration were substantially lower than after intravenous administration (<3% with peribulbar injection, <0.2% with balloon). Local administration should, therefore, decrease the risk of systemic toxic effects and may potentially decrease anterior segment toxic effects. No animal showed evidence of toxic effects, irritation, or pain to the eye or peribulbar tissues after local administration of carboplatin by external or dilated examination, but ocular toxic effects of locally administered carboplatin require further study. In a rabbit model, 10 µg of carboplatin injected into rabbit vitreous humor was the lowest dose that caused retinal toxic effects.\textsuperscript{17}

This work stimulated the development of a phase I/II clinical protocol. We are currently evaluating the toxic effects and efficacy of locally administered carboplatin in patients with retinoblastoma at New York Hospital, New York, NY.

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REFERENCES


A look at the past . . .

Dr. DOR made a few . . . remarks on the treatment of detached retina. The patient must remain in bed, if necessary for months. Heurteloup was applied to the temple daily. The sclera was cauterized, at first twice, and later once a week, over the point of detachment. A Paquelin cautery was used.