

**OBJECTIVE:** To determine whether intra-arterial chemotherapy is safe and effective in advanced intraocular retinoblastoma.

**DESIGN:** Single-arm, prospective registry from May 30, 2006, to May 30, 2010, at an ophthalmic oncology referral center with ambulatory care. A total of 95 eyes of 78 patients with unilateral or bilateral retinoblastoma were treated. The intervention was selective catheterization of the ophthalmic artery and injection of chemotherapy, usually melphalan with or without topotecan. Drug dosage was determined by age and angioanatomy. The main outcome measures were procedural success, event-free (enucleation or radiotherapy) ocular survival, and ocular and extraocular complications.

**RESULTS:** Catheterization succeeded in 98.5% of procedures. There were 289 chemotherapy injections (median, 3 per eye). The Kaplan-Meier estimates of ocular event-free survival rates at 2 years were 70.0% (95% confidence interval, 57.9%-82.2%) for all eyes, 81.7% (95% confidence interval, 66.8%-96.6%) for eyes that received intra-arterial chemotherapy as primary treatment, and 58.4% (95% confidence interval, 39.5%-77.2%) for eyes that had previous treatment failure with intravenous chemotherapy and/or external beam radiation therapy. There were no permanent extraocular complications.

**CONCLUSION:** Our experience suggests that intra-arterial chemotherapy is safe and effective in the treatment of advanced intraocular retinoblastoma.

Comment: This study by the experts in this technique shows that the procedure can be done, but success rates may differ in less experienced hands. The best success was for eyes that received intra-arterial chemotherapy as a primary treatment. This treatment was not nearly as successful (only 58.4%) for eyes that had previous treatment failure and therefore use as a rescue therapy requires more analysis.

Novel retinoblastoma treatment avoids chemotherapy: the effect of optimally timed combination therapy with angiogenic and glycolytic inhibitors on LH(BETA)T(AG) retinoblastoma tumors.


**PURPOSE:** The purpose of this study was to evaluate the effect of optimally timed combination treatment with angiogenic and glycolytic inhibitors on tumor burden, hypoxia, and angiogenesis in advanced retinoblastoma tumors.

**METHODS:** LH(BETA)T(AG) mice (n=30) were evaluated. Mice were divided into 5 groups (n=6) and received injections at 16 weeks of age (advanced tumors) with a) saline, b) anecortave acetate (AA), c) 2-deoxyglucose (2-DG), d) AA +2-DG (1 day post-AA treatment), or e) AA +2-DG (1 week post-AA treatment). Eyes were enucleated at 21 weeks and tumor sections were analyzed for hypoxia, angiogenesis, and tumor burden.

**RESULTS:** Eyes treated with 2-DG 1 day post-AA injection showed a 23% (P=0.03) reduction in tumor burden compared with 2-DG alone and a 61% (P < 0.001) reduction compared with saline-treated eyes. Eyes treated with 2-DG 1 week post-AA injection showed no significant decrease in tumor burden compared with 2-DG alone (P = 0.21) and a 56% (P < 0.001) decrease in comparison with saline-treated eyes. 2-DG significantly reduced the total density of new blood vessels in tumors by 44% compared to saline controls (P < 0.001), but did not affect the density of mature vasculature.

**CONCLUSIONS:** Combination therapy with angiogenic and glycolytic inhibitors significantly enhanced tumor control. Angiogenic and glycolytic inhibitors may have significant potential as alternative therapies for treating children with retinoblastoma.

Comment: This study was done in mice, so clinical trials in humans would be needed to confirm the effect, but it may offer another treatment for Rb that could avoid chemo and radiation. The side effects of these new agents would also have to be investigated.
Occurrence of sectoral choroidal occlusive vasculopathy and retinal arteriolar embolization after superselective ophthalmic artery chemotherapy for advanced intraocular retinoblastoma.


BACKGROUND: Superselective ophthalmic artery chemotherapy (SOAC) has recently been proposed as an alternative to intravenous chemoreduction for advanced intraocular retinoblastoma. In this study, we report on the vascular adverse effects observed in our initial cohort of 13 patients.

METHODS: The charts of 13 consecutive patients with retinoblastoma who received a total of 30 injections (up to 3 injections of a single agent per patient at 3-week interval) of melphalan (0.35 mg/kg) in the ophthalmic artery between November 2008 and June 2010 were retrospectively reviewed. RetCam fundus photography and fluorescein angiography were performed at presentation and before each injection. Vision was assessed at the latest visit.

RESULTS: Enucleation and external beam radiotherapy could be avoided in all cases but one, with a mean follow-up of 7 months. Sectoral choroidal occlusive vasculopathy leading to chorioretinal atrophy was observed temporally in 2 eyes (15%) 3 weeks to 6 weeks after the beginning of SOAC and retinal arteriolar emboli in 1 eye 2 weeks after injection. There was no stroke or other clinically significant systemic side effects except a perioperative transient spasm of the internal carotid artery in one patient. Vision ranged between 20/1600 and 20/32 depending on the status of the macula.

CONCLUSION: Superselective ophthalmic artery chemotherapy was effective in all patients with no stroke or other systemic vascular complications. Unlike intravenous chemoreduction, SOAC is associated with potentially sight-threatening adverse effect. Further analysis of the risks and benefits of SOAC will define its role within the therapeutic arsenal. Meanwhile, we suggest that SOAC should be given in one eye only and restricted to advanced cases of retinoblastoma, as an alternative to enucleation and/or external beam radiotherapy.

Comment: Fortunately no patient had a sustained systemic complication but there was one case of carotid spasm. Three patients had sight-threatening complications, however 12/13 avoided enucleation and/or radiation with this treatment.

Pre-enucleation chemotherapy for eyes severely affected by retinoblastoma masks risk of tumor extension and increases death from metastasis.


PURPOSE: Initial response of intraocular retinoblastoma to chemotherapy has encouraged primary chemotherapy instead of primary enucleation for eyes with clinical features suggesting high risk of extracocular extension or metastasis. Upfront enucleation of such high-risk eyes allows pathologic evaluation of extracocular extension, key to management with appropriate surveillance and adjuvant therapy. Does chemotherapy before enucleation mask histologic features of extracocular extension, potentially endangering the child’s life by subsequent undertreatment?

METHODS: We performed retrospective analysis of 100 eyes with advanced retinoblastoma enucleated with, or without, primary chemotherapy, in Beijing Tongren Hospital, retrospectively, from October 31, 2008. The extent of retinoblastoma invasion into optic nerve, uvea, and anterior chamber on histopathology was staged by pTNM classification. The treatment groups were compared for pathologic stage and disease-specific mortality.

Results: Children who received chemotherapy before enucleation had lower pTNM stage than primarily enucleated children (P = .01). Five patients who received pre-enucleation chemotherapy died as a result of extension into brain or metastasis. No patients who had primary enucleation died. For children with group E eyes, disease-specific survival (DSS) was lower with pre-enucleation chemotherapy (n = 45) than with primary enucleation (n = 37; P = .01). Enucleation longer than 3 months after diagnosis was also associated with lower DSS (P < .001).

CONCLUSION: Chemotherapy before enucleation of group E eyes with advanced retinoblastoma downstaged pathologic evidence of extracocular extension, and increased the risk of metastatic death from reduced surveillance and inappropriate management of high-risk disease, if enucleation was performed longer than 3 months after diagnosis.

Comment: In this important paper the data suggest that children with advanced retinoblastoma have a greater chance of metastasis and death if they receive chemotherapy before enucleation. Parents of
children with advanced Rb should know the risks. Rapid enucleation of group E eyes may give the greatest chance of disease-free survival. More data would be helpful in this decision-making.

Rabbit model of retinoblastoma.


We created a rabbit model of retinoblastoma and confirmed the tumor clinically and histopathologically. Seventeen New Zealand rabbits were immunosuppressed with cyclosporin A at doses of 10-15 mg/kg. At day 3, the animals received a 30 μl subretinal injection of 1 x 10⁶ cultured WERI retinoblastoma cells. Digital fundus images were captured before euthanasia, and the eyes were submitted for histopathology. Retinoblastoma cells grew in all the inoculated eyes and established a tumor under the retina and/or in the vitreous. New blood vessels in the tumor were observed starting at week 5. Cuffs of viable tumor cells surrounded the blood vessels with regions of necrosis present at 70-80 μm from nutrient vessels.

Occasional tumor seeds in the vitreous histologically exhibited central necrosis. This rabbit model demonstrated similar fundus appearance and pathologic features to human retinoblastoma and may be used as a model to test various routes of drug delivery for retinoblastoma.

Comment: This is a very useful new model to study treatments for Rb.

Lighting a candle in the dark: advances in genetics and gene therapy of recessive retinal dystrophies.


Nonsyndromic recessive retinal dystrophies cause severe visual impairment due to the death of photoreceptor and retinal pigment epithelium cells. These diseases until recently have been considered to be incurable. Molecular genetic studies in the last two decades have revealed the underlying molecular causes in approximately two-thirds of patients. The mammalian eye has been at the forefront of therapeutic trials based on gene augmentation in humans with an early-onset nonsyndromic recessive retinal dystrophy due to mutations in the retinal pigment epithelium-specific protein 65kDa (RPE65) gene. Tremendous challenges still lie ahead to extrapolate these studies to other retinal disease-causing genes, as human gene augmentation studies require testing in animal models for each individual gene and sufficiently large patient cohorts for clinical trials remain to be identified through cost-effective mutation screening protocols.

Comment: This is a truly excellent review of gene therapy for retinal dystrophies. It is very well written and the illustrations are superb. It explains vision at a cellular level, as well as discussing treatment.

Gene therapy for Leber's congenital amaurosis is safe and effective through 1.5 years after vector administration.


The safety and efficacy of gene therapy for inherited retinal diseases is being tested in humans affected with Leber's congenital amaurosis (LCA), an autosomal recessive blinding disease. Three independent studies have provided evidence that the subretinal administration of adeno-associated viral (AAV) vectors encoding RPE65 in patients affected with LCA2 due to mutations in the RPE65 gene, is safe and, in some cases, results in efficacy. We evaluated the long-term safety and efficacy (global effects on retinal/visual function) resulting from subretinal administration of AAV2-hRPE65v2. Both the safety and the efficacy noted at early timepoints persist through at least 1.5 years after injection in the three LCA2 patients enrolled in the low dose cohort of our trial. A transient rise in neutralizing antibodies to AAV capsid was observed but there was no humoral response to RPE65 protein. The persistence of functional amelioration suggests that AAV-mediated gene transfer to the human retina does not elicit immunological responses which cause significant loss of transduced cells. The persistence of physiologic effect supports the possibility that gene therapy may influence LCA2 disease progression. The safety of the intervention and the stability of the improvement in visual and retinal function in these subject support the use of AAV-mediated gene augmentation therapy for treatment of inherited retinal diseases.

Comment: This study reports that the effects of subretinal gene therapy in 3 patients with RPE65 LCA were long lasting and safe, which is great news for patients with this type of LCA.
Safety and efficacy of subretinal readministration of a viral vector in large animals to treat congenital blindness.


Leber's congenital amaurosis (LCA) is a group of severe inherited retinal degenerations that are symptomatic in infancy and lead to total blindness in adulthood. Recent clinical trials using recombinant adeno-associated virus serotype 2 (rAAV2) successfully reversed blindness in patients with LCA caused by RPE65 mutations after one subretinal injection. However, it was unclear whether treatment of the second eye in the same manner would be safe and efficacious, given the potential for a complicating immune response after the first injection. Here, we evaluated the immunological and functional consequences of readministration of rAAV2-hRPE65v2 to the contralateral eye using large animal models. Neither RPE65-mutant (affected: RPE65(-/-)) nor unaffected animals developed antibodies against the transgene product, but all developed neutralizing antibodies against the AAV2 capsid in sera and intraocular fluid after subretinal injection. Cell-mediated immune responses were benign, with only 10 animals in the study developing a persistent T cell immune response to AAV2, a response that was mediated by CD4(+) T cells. Sequential bilateral injection caused minimal inflammation and improved visual function in affected animals. Thus, subretinal readministration of rAAV2 in animals is safe and effective, even in the setting of preexisting immunity to the vector, a parameter that has been used to exclude patients from gene therapy trials.

Comment: This excellent study demonstrates that in dogs that have received subretinal injection treatment with AAV in one eye, injecting the second eye is safe and effective. There is an antibody response to the AAV2 capsid, and 1/10 animals had a T cell response, but these were not clinically significant.

Mutations in chaperonin-like BBS genes are a major contributor to disease development in a multiethnic Bardet-Biedl syndrome patient population.


BACKGROUND: Bardet-Biedl syndrome is a pleiotropic disorder with 14 BBS genes identified. BBS1, BBS2, BBS4, BBS5, BBS7, BBS8, and BBS9 form a complex called the BBSome, which is believed to recruit Rab8(GTP) to the primary cilium and promote ciliogenesis. The second group, the chaperonin-like proteins BBS6, BBS10, and BBS12, have been defined as a vertebrate-specific branch of the type II chaperonin superfamily. These may play a role in the regulation of BBSome assembly.

METHODS AND RESULTS: Using sequence analysis, the role of BBS6, 10 and 12 was assessed in the patient population comprising 93 cases from 74 families. Systemic and ocular phenotypes were defined. In the study, chaperonin-like BBS gene mutations accounted for the disease in approximately 36.5% of BBS families. A total of 38 different non-polymorphic exonic sequence variants were identified in 40.5% of BBS families (41.9% cases), of which 26 were novel (68%). Six cases had mutations present in more than one chaperonin-like BBS gene. One case with four mutations in BBS10 had a phenotype of overall greater severity. The phenotypes observed were beyond the classic BBS phenotype as they overlapped with characteristics of MKKS (congenital heart defect, vaginal atresia, hydrometrocolpos, cryptorchidism), as well as Alström syndrome (diabetes, hearing loss, liver abnormalities, endocrine anomalies, cardiomyopathy).

CONCLUSIONS: While overlap between the MKKS and BBS phenotypes has previously been reported for cases with BBS6 mutations, we also observed MKKS phenotypes involving BBS10 and BBS12 and Alström-like phenotypes associated with mutations in BBS1, BBS2, BBS6, BBS7, BBS9, BBS10 and BBS12 for the first time.

Comment: This is an excellent study with a large group of BBS patients. This disorder has such unusual findings—extra digits, obesity, retinitis pigmentosa—that it was difficult to understand how so many different genes could cause the same phenotype until the BBSome was discovered. This is a protein complex involved in intracellular trafficking which requires the protein product of multiple BBS genes to form correctly. This paper shows that other related genes cause a large percentage of BBS, and that there is phenotypic overlap with other syndromes such as MKKS and Alstrom syndrome.