Postmortem vitreous bevacizumab levels of an infant treated for retinopathy of prematurity

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We report the vitreous concentration of bevacizumab after injection for the treatment of retinopathy of prematurity (ROP). A premature neonate diagnosed with type 1 ROP was treated in both eyes with 0.625 mg intravitreal bevacizumab injection at 32 weeks' postconceptual age. Eleven weeks later there was complete regression clinically, but the patient died. Vitreous samples taken at autopsy revealed a bevacizumab vitreous concentration of 41.57 ng/ml. Histopathology of the retina showed residual preretinal neovascularization. Bevacizumab elimination from the infant vitreous is similar to that of adults, and, although complete regression was clinically apparent, it was not confirmed histopathologically.

Case Report

A former premature boy of 23 weeks' postconceptual age (PCA) and birth weight of 610 g was managed in the pediatric intensive care unit of Akron Children's Hospital because of respiratory failure and intraventricular hemorrhage. He was diagnosed with chronic bronchopulmonary dysplasia, patchy necrotizing bronchiolitis, and anemia. His first ROP screening examination at 31 weeks' PCA revealed immature retinas in zone II in each eye. At age of 32 weeks' PCA he developed stage 2 zone II ROP in each eye that progressed to stage 3 zone II with plus disease (type 1) ROP in each eye. The patient was treated with an intravitreal injection of 0.625 mg (0.02cc) bevacizumab in each eye. Regression of plus and stage 3 occurred in each eye within 1 week of injection. Regression of ROP continued during weekly examinations and at 43 weeks of age, complete regression with avascular retina temporally for 4-6 clock hours in each eye was left. At 44 gestational weeks, the patient developed severe and irreversible pulmonary failure. The family decided to withhold further treatment.

The patient deceased at 44 weeks' PCA, 11 weeks after intravitreal bevacizumab injection. An autopsy included examination of both eyes; 0.5 ml of vitreous samples were obtained under sterile conditions, and enucleation of the globes was then performed 14 hours after the patient's decease. The vitreous samples were stored in regular ice during transport to laboratory and sent for analysis of bevacizumab levels.

Histopathology revealed pathological neovascularization in posterior zone 2 consisted of neovascular tufts that are beneath the nerve fiber layer (NFL), implying a regressed stage 2 and a neovascular sprouting on the optic disc extending beyond the NFL that could imply neovascularization of the optic disk (Figure 1). Bevacizumab vitreous levels were quantified by enzyme-linked immunosorbent assay (ELISA) in triplicates immediately after obtaining the vitreous samples and 4 weeks later and were found to be 41.57 ng/ml and 24.47 ng/ml, respectively. Systemic levels of bevacizumab were not obtained.

Discussion

Retinal ablative therapy is indicated for type 1 ROP, defined as zone 1, any stage ROP with plus disease, for zone 1, stage 3 ROP without plus disease, or for zone 2, stage 2 or 3 ROP with plus disease. The most common treatments are retinal cryoablasion and laser therapy; most ophthalmologists use the latter.[1-3] IVT injection of bevacizumab has been increasingly reported as a treatment of ROP[4,5]; however, its long-term effects and safety in this context remain unknown and somewhat controversial.[6] To our knowledge, this is the first case report that describes the level of bevacizumab in the vitreous humor of an infant eye after injection. A literature review did not yield any reports of IVT levels or pharmacokinetics of bevacizumab in the infant eye.

Eleven weeks after IVT injection of bevacizumab we found that bevacizumab levels were below detectable levels, at 41.57 ng/ml. The value is below the lowest standard in the ELISA kit used but consistent with other reports.[7-9]

Current understanding of intravitreal pharmacokinetics is currently based on the drug's behavior in adult and animal eyes. Previous reports in nonvitrectomized adult eyes found the aqueous half-life after a single injection of IVT bevacizumab to be 9.83 days.[10] After IVT injection of 1.25 mg/ml of bevacizumab, its concentration peaked on postinjection day 1 with a mean concentration of 33.3 μg/ml and dropped off to $< 1\mu$g/ml at day 51.[10] Other reports found the half-life in the adult vitreous to be 2.5-6.7 days and the vitreous level 48 hours and 4 weeks after injection of 1.25 mg/ml was 0.6625 mg/ml (53% of the

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Submitted April 19, 2016.
Revision accepted July 7, 2016.
Published online October 31, 2016.
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J AAPOS 2016;20:537-539.

1091-8531/$36.00
http://dx.doi.org/10.1016/j.jaapos.2016.07.229

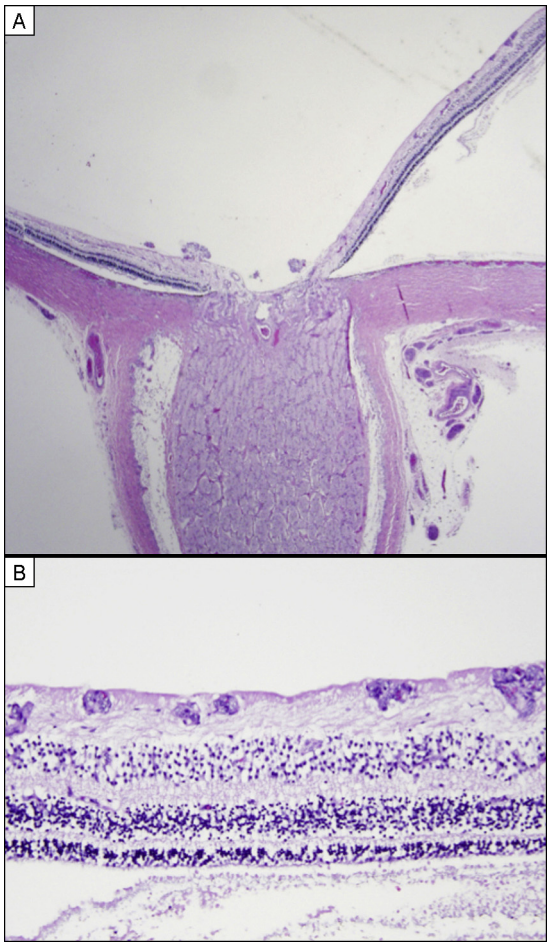

FIG 1. A, Low-magnification histological section of the eye showing the pathological neovascularization in posterior zone II (original magnification ×10). Note that these neovascular tufts are beneath nerve fiber layer (NFL) implying a regressed stage 2. Also note the neovascular sprouting on the optic disk extending beyond NFL that could imply NVD. B, High-magnification histological section of posterior zone II showing pathological neovascularization consisted of pathological epithelial cells (original magnification ×40). Note that the vessels are not forming a retinal ridge and are not protruding into the vitreous implying a regressed neovascularization of zone II.

loading dose) and 0.002 mg/ml (0.16% of loading dose), respectively.[7-9] Bevacizumab has been detected in the vitreous at 84 days via ELISA and 101 days via Western blot analysis after a single 1.25 mg/ml injection in adults, with vitreous levels of 149.37 ng/ml, 107.26 ng/ml and19.12 ng/ml after 1, 4 and 8 weeks from injection, respectively.[8] In another study, vitreous samples were taken through vitrectomy from 11 patients on day 2 through day 101.[9] Bevacizumab levels ranged from 2.63 ng/ml to 165 μg/ml. The peak concentration was observed on the second day after injection. At week 11 after injection, bevacizumab level was 21.9 ng/ml.[9]

In our case we found a level of vitreous bevacizumab of 41.57 ng/ml 11 weeks after injection of 0.625 mg. This level is consistent with levels found in other studies.[7-9] The low level could be accounted for by the natural pharmacokinetic curve of vitreous bevacizumab found by Zhu and collegues.[9]

The histopathology findings in this report have special significance because they show the regression of the pathological blood vessels in zone 2, which correlates with the clinical findings. On the other hand, histopathology findings show that even after an impressive regression of the disease, some neovascular tufts persisted beneath the NFL at the area of regression and there was some neovascular sprouting on the optic disc The implications of the these findings are that the disease can be subtly active and undetected clinically after treatment with single injection of bevacizumab and impressive clinical regression. After treatment with bevacizumab, a thorough and detailed examination should be performed for any pathological finding and accordingly to adjust the treatment with bevacizumab or switch to laser therapy.

Our findings suggest the rate of elimination of bevacizumab from the infant vitreous may not differ from that of adults. With the increasing use of intravitreal injections of anti-VEGF substances to treat ROP, information on their intraocular elimination kinetics will be important in order to optimize injection dosing and reinjection intervals. More comprehensive studies of bevacizumab pharmacokinetics in infants and patients with ROP are necessary and will lead to increased standardization of intravitreal treatment protocols in children.

Literature Search

The authors conducted a literature search on March 25, 2016, using PubMed and Cochrane search engines, with results restricted to English-language publications with the following keywords: *bevacizumab*, *Avastin*, *retinopathy of prematurity*, and *pharmacokinetics*.

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Refractive outcomes following the treatment of retinopathy of prematurity in the anti-VEGF era: a literature review

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A growing body of evidence indicates that antivascular endothelial growth factor (VEGF) therapy is effective in the treatment of retinopathy of prematurity (ROP). We conducted a comprehensive literature review on refractive outcomes of anti-VEGF treatments compared to laser treatment or a combination of laser therapy and anti-VEGF injections. Of the 9 studies analyzed, the final mean refractive error was myopic in 3 studies (37%) with IVB monotherapy, 7 studies (87.5%) with laser photocoagulation, and 1 study (50%) with combined therapy. In comparing IVB with laser monotherapy, 6 of 7 studies (86%) reported that final refractive error was significantly more myopic (>1 D) after laser treatment. No study was graded as high quality, and only a single article provided moderate quality of evidence.

Currently, laser photocoagulation of the avascular retina is the standard treatment for eyes with high-risk retinopathy of prematurity (ROP). Severe myopia has been reported as a possible adverse effect of laser photocoagulation.[1] Recent studies have shown less myopia after intravitreal bevacizumab (IVB) injections in eyes with ROP, which seems to contradict reports of the higher prevalence of myopia in patients who have undergone laser photocoagulation or cryotherapy.[1-4] This study reviewed the current literature regarding the refractive outcomes of eyes with ROP that underwent anti-VEGF therapy compared to those treated with laser or combined laser and anti-VEGF treatment.

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Submitted April 8, 2016.
Revision accepted September 14, 2016.
Published online October 31, 2016.

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J AAPOS 2016;20:539-540.

1091-8531/$36.00
http://dx.doi.org/10.1016/j.jaapos.2016.09.013

Methods

A comprehensive search of PubMed and Scopus databases for English-language publications on ROP covering the period January 2005 to April 2016 was conducted by 2 authors (JK, KGF). Search terms included the following: *retinopathy of prematurity*, *ROP*, *anti-VEGF*, *intravitreal bevacizumab*, *ranibizumab*, *aflibercept*, *refractive error*, *refraction*, *myopia*, and *hyperopia*. Original articles comparing anti-VEGF treatment with a control group were extracted. Case reports and noncomparative case series were excluded. Only articles reporting refractive outcomes were analyzed. The quality of evidence was rated by 2 authors (KGF, JK) according to the Scottish Intercollegiate Guidelines Network (SIGN) guidelines[5] and GRADE[6] criteria, considering the refractive error as the main outcome measure.

Results

Of 3,568 articles retrieved, 122 articles discussed anti-VEGF therapy. Of these, 19 reported refractive outcomes of intravitreal anti-VEGF injections in ROP, and 9 studies met inclusion criteria (eTable 1).[1-4,7-11] All studies used bevacizumab for intravitreal injections; all but 2 were retrospective.[1,2] With IVB monotherapy, final mean refractive error was emmetropic (range, −1 to +1 D) in 5 studies and myopic (≤−1 D) in the 3 remaining reports. With laser monotherapy, final mean refractive error was hyperopic (≥+1 D) in 1 study and myopic in the remaining reports. In patients treated with combined IVB and laser treatment group, final mean refractive error was emmetropic in one study and myopic in another.

Comparison of IVB with Laser Monotherapy

Seven studies compared IVB with laser treatment alone. Six studies reported that final refractive error was significantly more myopic after laser treatment.[1-4,8,9] In these reports, the mean final refractive error ranged from +0.42 to −3.70 D for the IVB group and from −4.41 to −10.1 D for the laser group. The difference of the refractive error between the two groups was 2.82–7.08 D.

One study reported similar refractive outcomes in IVB and laser groups (−1.53 and −1.71 D, resp.).[10]

Comparison of IVB and Combined IVB and Laser Treatment

One study compared IVB monotherapy with combined IVB and laser treatment.[7] The authors reported greater