Acta Ophthalmologica

Intravitreal low-dosage bevacizumab for retinopathy of prematurity

Björn C. Harder, Stefan von Baltz, Jost B. Jonas and Frank C. Schlichtenbrede

Department of Ophthalmology, Medical Faculty Mannheim, Ruprecht-Karls-University Heidelberg, Heidelberg, Germany

ABSTRACT.

Purpose: **To report on the therapeutic effect of intravitreal low-dose bevacizumab for treatment for retinopathy of prematurity (ROP).**

Methods: **The single-centre retrospective, non-comparative case series study included all infants who consecutively underwent intravitreal injection of 0.375 mg bevacizumab (0.03 ml) under light sedation in topical anaesthesia as therapy of ROP in zone I or zone II.**

Results: **The clinical charts of 29 patients (57 eyes) with a median birth weight of 630 g (range: 290–1390 g) and median gestational age of 25 + 1 weeks (range: 23 + 1–30 weeks) were reviewed. Six children (12 eyes) were graded as ROP with zone I retinopathy and plus disease. The 23 remaining infants had extraretinal neovascularizations in zone II or partly zone I. The intravitreal bevacizumab injection was injected at a median age of 12 + 1 weeks (range: 7 + 4–21 + 4), the median follow-up was 4.2 months (range: from 3 days to 45.1 months). In all eyes treated, a regression of plus disease occurred within two to six days, retinal neovascularizations regressed within 2–3 weeks and pupillary rigidity improved. None except one child in exceptionally bad general health conditions needed a second intravitreal bevacizumab injection. In none of the infants, any ophthalmologic side-effects of the bevacizumab application were detected during the follow-up period.**

Conclusions: **The intravitreal injection of a low dose of 0.375 mg bevacizumab showed a high efficacy as treatment for ROP. The question arises whether the low dosage of bevacizumab as compared to the dosage of 0.625 mg bevacizumab may be preferred.**

Key words: bevacizumab – intra-ocular neovascularization – retinopathy of prematurity – vascular endothelial growth factor

Acta Ophthalmol. 2014: 92: 577–581

doi: 10.1111/aos.12266

Introduction

Retinopathy of prematurity (ROP) is a retinal vascular disease that affects premature infants. Its incidence of 27–40% in premature infants with a birth weight of <1500 g did not change markedly over the past twenty years despite intensive efforts and improvements in neonatal care (Larsson et al. 2002; Lad et al. 2008). Although many advances in understanding and managing the disease have been achieved the first description of the disease by Terry 1942; ROP is still a major cause of blindness in children in the developed and developing world (Terry 1942; Gilbert et al. 1997; Aslam et al. 2009; Murakami et al. 2010; Stefánsson 2011; Augestad et al.2012; Boonstra et al. 2012). Laser treatment has been regarded as the 'gold standard' for therapy of ROP, despite its inherent problems such as irreversible scar formation in the peripheral and midperipheral retina and choroid and potentially a marked myopization (The Early Treatment for Retinopathy of Prematurity Cooperative Group 2003; Fledelius & Jensen 2011). In addition, the rate of unfavourable outcomes as reported by the Early Treatment for Retinopathy of Prematurity Cooperative Group with 14.5% is still relatively high although it dropped from 19.5% after introduction of early laser treatment in eyes with zone 1 retinopathy. Other limitations of laser therapy of ROP include the necessity of general anaesthesia, which carries the risk of prolonged intubation postoperatively; and the necessity of clear optic media to allow the laser beam to reach the peripheral retina. In view of these limitations of laser therapy for ROP, intravitreal injection of bevacizumab has been examined as an alternative to the current laser therapy (Mintz-Hittner & Kuffel 2008; dell'Omo et al. 2008; Harder et al. 2011; Mintz-Hittner et al. 2011; Akkoyun et al. 2012). The use of bevacizumab was based on the role vascular endothelial growth factor (VEGF) plays in the pathogenesis of ROP (Nonobe et al. 2009). Most study centres reporting on the use of bevacizumab for ROP applied the high dosage of 0.625 mg bevacizumab. Because the risk of potential systemic side-effects of a temporary blockade of VEGF in the fast growing body of a prematurely born infant has not been sufficiently explored yet and because the risk of systemic side-effects of a drug admin-

istered locally may depend on its dosage applied, we performed this study to report on the clinical experience gathered in all infants receiving the low dosage of 0.375 mg bevacizumab for the therapy of ROP within the study period. In a cumulative manner, we included all infants who ever received bevacizumab in our hospital into the present report, which thus includes some infants for whom earlier data on an individually shorter follow-up have been presented in previous reports (Harder et al. 2011, 2012, 2013).

Methods

For the last 20 years, our department has been the screening and referral centre for ROP for the paediatric departments of the university hospitals of Mannheim and Heidelberg and for other regional neonatal centres. According to The Early Treatment for Retinopathy of Prematurity Cooperative Group (2003) with its revised indications for the treatment for ROP, we started in 2008 to offer parents of infants with type 1 ROP (zone I, any stage with plus disease; zone I stage 3 without plus disease; and zone II stages 2 or 3 with plus disease) the choice between standard laser treatment or the intravitreal application of bevacizumab. Before obtaining informed consent from the parents, we stressed the fact that the intravitreal injection bevacizumab was an experimental off-label therapy with no long-term results. The diagnosis of ROP was based on the revisited guidelines of the International Committee for the Classification of Retinopathy of Prematurity ICROP (International Committee for the Classification of Retinopathy of Prematurity 2005). We did not treat infants with zone I prethreshold ROP in stages 1 and 2 without plus disease because this type 2 ROP has been reported to have a relatively low conversion rate into type 1 ROP (Christiansen et al. 2010).

Our retrospective non-randomized interventional comparative study included all children who were consecutively treated with an intravitreal injection of bevacizumab (Avastin®; Genentech Inc., San Francisco, CA, USA) for type 1 ROP disease in zone II or zone I. It was a retrospective analysis of clinical data obtained during routinely taking care of the children. The Ethics Committee II of the Medical Faculty Mannheim of the Ruprecht-Karls-University Heidelberg approved the study (# 2012-613R-MA).

The intravitreal bevacizumab injection was injected under sterile conditions using sterile gloves, drapes and speculum after rigorously flushing of the conjunctival cul-de-sac with 5% povidone-iodine 5% (Betadine®; Alcon, Ft Worth, TX, USA). The dosage of bevacizumab was 0.375 mg in 0.03 ml. Due to the small volume of the injection, an anterior chamber paracentesis was not performed. At 1.5 mm posterior to the limbus in the temporal inferior quadrant, the 30-gauge needle was passed through the sclera in an oblique way to minimize a reflux. Without tracheal intubation, the operation was performed in light sedation under topical anaesthesia using oxybuprocainhydrochlorid eye drops. Postoperatively, we applied an steroid–antibiotic eye drop combination of dexamethasone, neomycin and polymyxin B (Isoptomax AT®; Alcon, Ft Worth, TX, USA) four times and atropine (0,5%) eye drops (Atropin POS 0.5%; Ursapharm Arzneimittel GmbH, 66129 Saarbrücken, Germany) twice a day for 1 week. The surgical procedure was performed by two experienced surgeons (FCS, BCH).

The postoperative ophthalmological examinations were performed without anaesthesia or sedation. After instillation of medical mydriasis, the children were held by a nurse, topical anaesthesia was performed by oxybuprocainhydrochlorid eye drops, a lid speculum was inserted, and the fundus including its periphery was examined using a binocular ophthalmoscope and squint hooks to slightly turn the globe into the direction of examination. Potential systemic side-effects of the bevacizumab therapy were assessed during the routine examinations during the surveillance in the neonatal care unit or by the local paediatricians. Due to the retrospective design of the study, systemic side-effects were not systematically searched for.

Results

The clinical charts of 57 eyes of 29 patients who were treated for type 1 ROP in zone II or zone I were reviewed. The median birth weight was 630 g (range: 290–1390 g), and the median gestational age was 25 + 1 weeks (range 23 + 1–30 weeks). Six children (12 eyes) were graded as ROP with zone I retinopathy and plus disease. The 23 remaining infants had retinal neovascularizations mostly in the posterior part of zone II partially overlapping with zone I. The intravitreal bevacizumab injection was injected at a median age of 12 + 1 weeks (range between 7 + 4 and 21 + 4), the median follow-up was 4.2 months (mean: 10.7 months; range from 3 days to 45.1 months). One of the first three infants in the study population underwent a primary laser treatment in one eye and received an intravitreal bevacizumab injection in the other eye in which a persistent tunica vasculosa lentis prevented a laser therapy of the fundus. Because the eye that had received laser therapy only continued to show neovascularization, the eye additionally received an intravitreal injection of bevacizumab 4 weeks after the laser therapy. All other infants received only intravitreal bevacizumab as solely treatment for ROP. For one child, we treated one eye only which had developed asymmetric ROP with massive preretinal neovascularizations for more than 120° in zone II with dilation of the temporal superior vessels and temporal inferior vessels, while the other eye remained stable in ROP stage 2.

In all eyes treated, we observed a regression of plus disease within 2–6 days after the intravitreal bevacizumab injection. Retinal and preretinal neovascularizations became less perfused and regressed within 2–3 weeks. Simultaneously, the rigidity of the pupil improved and a tunica vasculosa lentis if present at the time of injection regressed. The infant who showed the asymmetrical ROP with massive neovascularization for 120° eventually developed retinal scaring with macular ectopia and the retina remaining attached. None of the other eyes developed an unfavourable anatomical outcome after the single injection of bevacizumab. One child developed a recurrence of plus disease at 95 days after a first intravitreal injection of bevacizumab for therapy of zone 1 ROP with plus disease. The infant suffered from anaemia with a blood haemoglobin concentration of 6 mg/dl and a history of 26 blood transfusions

and tracheal intubation for 8 weeks after infection with parvovirus B19. After reinjecting bevacizumab, the plus disease disappeared again without signs of retinal traction. None of the other infants received a second intravitreal bevacizumab injection. Similar to the results of The Early Treatment for Retinopathy of Prematurity Cooperative Group (2003), we found an unfavourable outcome in the eye that received a laser treatment in zone I retinopathy. The eye developed a retinal fold with macular ectopia. The contralateral eye that showed a persistent tunica vasculosa lentis and that only received a single intravitreal bevacizumab injection did not develop any signs of retinal folds or macular ectopia.

We did not detect any ophthalmologic side-effects of the bevacizumab application in none of the infants neither during the surveillance in the neonatal care unit or during the follow-up period.

Discussion

Our single-centre retrospective, non-comparative cumulative case series study revealed that all eyes treated with a low dosage of 0.375 mg bevacizumab for therapy of type 1-ROP in zone I or zone II showed a regression of plus disease within 2–6 days, regression of the retinal neovascularizations within 2–3 weeks, and pupillary rigidity improved. None except one child in exceptionally bad general health conditions needed a second intravitreal bevacizumab injection. In none of the infants, any ophthalmologic side-effects of the bevacizumab application were detected during the follow-up period.

These results of our study agree with numerous studies on the general use of intravitreal bevacizumab for therapy of ROP (Mintz-Hittner & Kuffel 2008; dell'Omo et al. 2008; Mintz-Hittner et al. 2011). They confirm previous smaller-scaled investigations on the similar group of patients who had cumulatively been included in this ongoing study on the use of low-dosage bevacizumab for the therapy of ROP (Harder et al. 2011, 2012, 2013). If compared with findings of the studies using the high dosage of intravitreal bevacizumab, the results of our report suggest that the similar results in terms of regression of plus disease and retinal neovascularization in infants with ROP can be obtained using the low dosage of bevacizumab (Spandau 2013; Spandau et al. 2013).

The major concern of the use of bevacizumab for therapy of ROP is the unknown risk of systemic side-effects in these premature and usually frail infants in their third trimester of gestation. Angiogenesis plays a major role in the developing of most organs, particularly in these fast growing infants. Sato and colleagues recently found a significant increase in the serum concentrations of bevacizumab before and at 1 day, 1 week and 2 weeks after a total of 0.5 mg of intravitreal bevacizumab of 0 ng/ml, 195 ± 324 ng/ml, 946 ± 680 ng/ml and 1214 ± 351 ng/ml, respectively (Sato et al. 2012). The serum bevacizumab levels before and at 1 day and at 1 week after a total 1.0 mg of intravitreal bevacizumab were 0 ng/ml, 248 ± 174 ng/ml and 548 ± 89 ng/ml, respectively. This increase in the serum bevacizumab levels coincided with a significant decrease in the serum concentrations of VEGF. In addition, the serum concentration of bevacizumab was significantly and negatively correlated (correlation coefficient $r = -0.5$; $p = 0.01$) with the serum concentration of VEGF. Because systemic VEGF is needed for the normal development of the body, the study by Sato et al. and studies by others (Hoerster et al. 2013) show the potential risk of systemic side-effects of intravitreally administered bevacizumab in infants. In view of the dosage relationship between the serum concentration of bevacizumab and the serum concentration of VEGF, the lowest possible dosage of intravitreally applied bevacizumab may be used for infants with ROP. This may hold true even more since the study by Mintz-Hittner and colleagues suggested that the assessment of mortality of the intravitreal use of bevacizumab in premature children would need a study sample size of 2800 infants and that the assessment of local or systemic toxicity of intravitreal bevacizumab would require an even larger number of infants in the study. Because ROP is a relatively rare disease, one may assume that definite answers to questions on safety of the therapy will long have to be awaited for. In that context, it may only be prudent to use the lowest possible dosage of intravitreal bevacizumab to achieve the therapeutic goal.

Hu et al. (2012) reported recently on the recurrence of ROP in 17 eyes of nine infants after the intravitreal injection of bevacizumab. Five of these eyes progressed to retinal detachment requiring surgical repair. Mintz-Hittner and colleagues found a recurrence of ROP in 6% of 70 infants treated with intravitreal bevacizumab including two eyes with eventual retinal detachment and one eye with eventual macular dragging (Mintz-Hittner et al. 2011). Interestingly, only one infant in our cumulative series experienced a recurrence of the disease, which could finally be addressed by a second intravitreal low-dosage bevacizumab injection. That infant was in bad general conditions as shown by the anaemia, the high frequency of blood transfusions and the systemic parvovirus infection. All these examples demonstrate however that although the intravitreal bevacizumab treatment is effective in inducing regression of ROP in the majority of infants, the effect may be transient and few children may require retreatment. Future studies may address which factors are associated with the necessity of medical retreatment of ROP by intravitreal bevacizumab.

Potential limitations of our study should be mentioned. First, it was a retrospective, non-comparative cumulative case series study with all adherent disadvantages such as varying follow-up and lack of a control group. The infants with a short follow-up usually were referred from distant hospitals, which then took over the care of the infants. If, however, ROP had recurred or another complication had been detected, it would have been highly likely that these infants were rereferred to the treating third-referral centre. Second, the follow-up in general was too short to allow any statements on the long-term effect of the therapy. Because the active stage of ROP is a timely limited disease, which usually is prevalent in a relatively short time window of few weeks, the lack of long-term information may be more important for potential local and systemic side-effects than for the therapeutic efficacy of the therapy. For some children, the follow-up was even so short that it could only be determined

whether the therapy had an effect on the intra-ocular neovascularization and the appearance of the plus disease. We might have had excluded those infants with a very short follow-up period; however, we weighed the advantage of having all infants with the therapy included into the study higher than the disadvantage of a rather short follow-up for few children. Even in the child with the shortest follow-up of three days, we could see the plus disease disappear and the beginning regression of the neovascularization. Because however recent reports in the literature showed a late reactivation of ROP and consequent retinal detachment after the intravitreal bevacizumab injection (Hu et al. 2012), the wide range (3 days to 45.1 months) of follow-up in our study is a weakness in the study design and the rate of a reactivation of ROP and eventual retinal detachment may be falsely low. However, it is very likely that a retinal detachment would have been detected by the local ophthalmologists, and it is very likely that the local ophthalmologist would have referred the infant back to our hospital where the primary treatment had been performed. Third, the follow-up examinations for some of the children were performed by local ophthalmologists when the children had been discharged from the hospital. These ophthalmologists had less experience in examining infants with ROP than the authors of this study. The local ophthalmologists would however have detected an abnormality in the midperiphery of the fundus or a reoccurrence of the plus disease. Fourth, as also pointed out in the study by Mintz-Hittner and colleagues (Mintz-Hittner et al. 2011), the number of study participants was by far too low to allow any conclusions on the safety of the therapy. Due to the retrospective design of our study, systemic side-effects were not systematically searched for in this study. Fifth, we could have used ranibizumab instead of bevacizumab for the intravitreal medication, because ranibizumab has a shorter intra-ocular half-life (7.2 days versus 9.8 days in adults) (Krohne et al. 2008, 2012). To our knowledge however, most studies on the medical therapy of ROP such as the BEAT-ROP used bevacizumab (Mintz-Hittner et al. 2011) so that also we continued to use bevacizumab. Sixth, last but not least, it has to be emphasized that laser treatment is still the primary treatment for threshold ROP.

In conclusion, the intravitreal application of a low dosage of 0.375 mg bevacizumab showed a high efficacy as treatment for ROP. To avoid potential dosage-related systemic side-effects, the question may therefore arise whether the low dosage of bevacizumab may generally be preferred.

Financial Disclosures

Jost B. Jonas: Consultant for Allergan Inc.; Merck & Co., Inc.; Alimera Co.; Patent holder with CellMed AG, Alzenau, Germany.

References

Akkoyun I, Karabay G, Haberal N, Dagdeviren A, Yilmaz G, Oto S, Erkanli L & Akova YA (2012): Structural consequences after intravitreal bevacizumab injection without increasing apoptotic cell death in a retinopathy of prematurity mouse model. Acta Ophthalmol **90**: 564–570.

Aslam T, Fleck B, Patton N, Trucco M & Azegrouz H (2009): Digital image analysis of plus disease in retinopathy of prematurity. Acta Ophthalmol **87**: 368–377.

Augestad LB, Klingenberg O & Fosse P (2012): Braille use among Norwegian children from 1967 to 2007: trends in the underlying causes. Acta Ophthalmol **90**: 428–434.

Boonstra N, Limburg H, Tijmes N, van Genderen M, Schuil J & van Nispen R (2012): Changes in causes of low vision between 1988 and 2009 in a Dutch population of children. Acta Ophthalmol **90**: 277–286.

Christiansen SP, Dobson V, Quinn GE et al., Early Treatment for Retinopathy of Prematurity Cooperative Group (2010): Progression of type 2 to type 1 retinopathy of prematurity in the Early Treatment for Retinopathy of Prematurity Study. Arch Ophthalmol **128**: 461–465.

Fledelius HC & Jensen H (2011): Late subsequent ocular morbidity in retinopathy of prematurity patients, with emphasis on visual loss caused by insidious 'involutive' pathology: an observational series. Acta Ophthalmol **89**: 316–323.

Gilbert C, Rahi J, Eckstein M, O'Sullivan J & Foster A (1997): Retinopathy of prematurity in middle-income countries. Lancet **350**: 12–14.

Harder BC, von Baltz S, Jonas JB & Schlichtenbrede FC (2011): Intravitreal bevacizumab for retinopathy of prematurity. J Ocular Pharmacol Ther **27**: 623–627.

Harder BC, Von Baltz S, Schlichtenbrede FC & Jonas JB (2012): Early refractive outcome after intravitreal bevazicumab for retinopathy of prematurity. Arch Ophthalmol Vis Sci **130**: 800–801.

Harder BC, Schlichtenbrede FC, von Baltz S, Jendritza W, Jendritza B & Jonas JB (2013): Intravitreal bevacizumab for retinopathy of prematurity: refractive error results. Am J Ophthalmol **155**: 1119–1124.e1.

Hoerster R, Muether P, Dahlke C, Mehler K, Oberthür A, Kirchhof B & Fauser S (2013): Serum concentrations of vascular endothelial growth factor in an infant treated with ranibizumab for retinopathy of prematurity. Acta Ophthalmol **91**: e74–e75.

Hu J, Blair MP, Shapiro MJ, Lichtenstein SJ, Galasso JM & Kapur R (2012): Reactivation of Retinopathy of Prematurity after Bevacizumab Injection. Arch Ophthalmol **130**: 1000–1006.

International Committee for the Classification of Retinopathy of Prematurity (2005): The International Classification of Retinopathy of Prematurity Revisited. Arch Ophthalmol **123**: 991–999.

Krohne TU, Eter N, Holz FG & Meyer CH (2008): Intraocular pharmacokinetics of bevacizumab after a single intravitreal injection in humans. Am J Ophthalmol **146**: 508–512.

Krohne TU, Liu Z, Holz FG & Meyer CH (2012): Intraocular pharmacokinetics of ranibizumab following a single intravitreal injection in humans. Am J Ophthalmol **154**: 682–686.

Lad E, Nguyen T, Morton J & Moshfeghi DM (2008): Retinopathy of prematurity in the United States. Br J Opthalmol **92**: 320–325.

Larsson E, Carle-Petrelius B, Cernerud G, Ots L, Wallin A & Holmström G (2002): Incidence or ROP in two consecutive Swedish population based studies. Br J Ophthalmol **86**: 1122–1126.

Mintz-Hittner HA & Kuffel RR Jr (2008): Intravitreal injection of bevacizumab (avastin) for treatment of stage 3 retinopathy of prematurity in zone I or posterior zone II. Retina **28**: 831–838.

Mintz-Hittner HA, Kennedy KA & Chuang AZ, BEAT-ROP CooperativeGroup (2011): Efficacy of intravitreal bevacizumab for stage 3 + retinopathy of prematurity. N Engl J Med **364**: 603–615.

Murakami Y, Silva RA, Jain A, Lad EM, Gandhi J & Moshfeghi DM (2010): Stanford University Network for Diagnosis of Retinopathy of Prematurity (SUNDROP): 24-month experience with telemedicine screening. Acta Ophthalmol **88**: 317–322.

Nonobe NI, Kachi S, Kondo M, Takai Y, Takemoto K, Nakayama A, Hayakawa M & Terasaki H (2009): Concentration of vascular endothelial growth factor in aqueous humor of eyes with advanced retinopathy of prematurity before and after intravitreal injection of bevacizumab. Retina **29**: 579–585.

dell'Omo R, Kulkarni A & Franks WA (2008): Intravitreal and intracameral bevacizumab to treat neovascular complications of retinopathy of prematurity. Acta Ophthalmol **86**: 698–700.

Sato T, Wada K, Arahori H, Kuno N, Imoto K, Iwahashi-Shima C & Kusaka S (2012): Serum concentrations of bevacizumab (avastin) and vascular endothelial growth factor in infants with retinopathy of prematurity. Am J Ophthalmol **153**: 327–333.

Spandau U (2013): What is the optimal dosage for intravitreal bevacizumab for retinopathy of prematurity? Acta Ophthalmol **91**: e154.

Spandau U, Tomic Z, Ewald U, Larsson E, Akerblom H & Holmström G (2013): Time to consider a new treatment protocol for aggressive posterior retinopathy of prematurity? Acta Ophthalmol **91**: 170–175

Stefánsson E (2011): How to prevent retinopathy of prematurity. Acta Ophthalmol **89**: 3–4.

Terry TL (1942): Fibroplastic overgrowth of persistent tunica vasculosa lentis in infants born prematurely: report of cases – clinical aspects. Trans Am Ophthalmol Soc **40**: 262–284.

The Early Treatment for Retinopathy of Prematurity Cooperative Group (2003): Revised indications for treatment of retinopathy of prematurity: results of the early treatment for the retinopathy of prematurity randomised trial. Arch Ophthalmol **121**: 1684–1696.

Received on January 29th, 2013.
Accepted on July 27th, 2013.

Correspondence:
Jost B. Jonas, MD
Universitäts-Augenklinik
Theodor-Kutzer-Ufer 1-3
68167 Mannheim
Germany
Tel: + 49 621 383 6078
Fax: + 49 621 383 2077
Email: Jost.Jonas@medma.uni-heidelberg.de