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Premature Infants with Retinopathy Received Avastin Tend to have Lower Plasma BDNF Concentration and Delayed Mental Development

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Abstract

Objective: One of the most common causes of childhood blindness is Retinopathy of Prematurity (ROP). Avstin (bevacizumab) has been used in the treatment of ROP recently, but its long-term effect on cognitive and psychomotor development in patients is not known. Neurodevelopmental disorders are associated with deficits in Brain-Derived Neurotrophic Factor (BDNF) function. This study aimed to evaluate the neurological outcome and serum BDNF level of premature infants with ROP who have received Avastin versus Cryotherapy treatment.

Study Design: Premature infants with Birth Body Weight (BBW) < 1500 grams and gestational age(GA) < 30 wks who are back for Bayley Scales of Infant Development II (BSID II) evaluation at two years of corrected age were included in the initial survey. Only premature infants with ROP and had received either Avastin or cryotherapy were enrolled for the study. Medical records were reviewed. Blood BDNF contents were analyzed, and demographic data of both groups were investigated and compared.

Results: A total of 21 premature infants (35 eyes in total) who met the criteria were included in the study. There were six premature infants in the Avastin Group and 20 included in the Cryotherapy Group. No significant differences were found in GA, BBW, and Apgar scores at 1 and 5 minutes between groups. The MDI and PDI scores at 24 months of corrected age were 72.5±6.8 vs. 82.3±2.9 (p=0.213) and 68.4±4.7 vs. 72.6±2.3 (p=0.759) in Avastin and Cryotherapy group respectively. However, five infants of Group 1 and five infants of Group 2 (33% vs. 83%, p=0.038) revealed significant mental developmental delayed at 24 months of corrected age. The BDNF level of Group 1 was significantly higher compared to Group 2 (93.3±9.8 vs. 43.2±10.4, p=0.022).

Conclusion: Premature infants with ROP who received Avastin treatment had both a significantly higher ratio of mental developmental delay and a lowered BDNF level as compared to premature infants who received Cryotherapy for ROP treatment at corrected 24 months of age. A larger scale study is needed for a more conclusive result.

Keywords: Avastin; Brain Derived Neurotrophic Factor; Neurodevelopment; Retinopathy of Prematurity

Introduction

Very-low birth weight premature infants usually need a period of oxygen support after birth. However, the supplemental oxygen exposure will induce the Retinopathy of Prematurity (ROP). ROP is one of the most common causes of childhood blindness. Complications of ROP include losing sight, retinal folds, macula dragging, retinal tears and detachments, iris neovascularization, glaucoma, high myopia, photoreceptor dysfunction, visual field loss [1-5]. ROP includes 2 phases: 1 cessation of normal retinal vascular growth and vaso-obliteration, and II: retinal neovascularization [6-9]. Effective treatment for ROP such as cryotherapy and laser therapy has been widely used to prevent neovascularization.

Bevacizumab (Avastin) is a humanized anti-VEGF monoclonal antibody that has been used for proliferative eye diseases such as diabetic retinopathy and age-related macular degeneration to inhibit intraocular neovascularization. Furthermore, Avastin has applied in the treatment of ROP in recent years [10]. Although Avastin is given via intravitreal administration, there are also concerns that it may have a systemic effect and cause a long-term detrimental effect on neurodevelopment in premature infants.

Brain-Derived Neurotrophic Factor (BDNF) is one of the neurotrophin family of growth factors that plays a vital role in the growth of new neurons and synapses and promote survival of neuronal populations [11-15]. Neurodevelopmental disorders are associated with deficits in BDNF function [16]. Decreased serum BDNF level can be a marker of aberrant neurodevelopment in preterm infants [17]. This study aimed to evaluate the correlation between neurodevelopment outcome and the serum BDNF level of premature infants with ROP who had received intravitreal Avastin versus cryotherapy treatment.

Patients and Methods

Preterm infants with Gestational Age (GA) <30 weeks and Birth Body Weight (BBW) < 1500 grams who required ROP treatment during neonatal hospitalization were included in the study while they are back for the neurodevelopmental follow-up at 24 months' correct age. Infants with congenital anomalies were excluded.

As ROP in itself is an independent predictor of long-term neurodevelopment and because we were interested in the long-term effect of Avastin, we divided participants according to whether they had received this specific treatment of ROP and those who received cryotherapy. The ROP screening followed the revised US screening guidelines presented in 2006. In brief, infants with a BBW of less than 1500 gm or GA of 30 weeks or less, as well as a BBW between 1500 and 2000 gm or GA of greater than 30 weeks with an unstable clinical course should have a retinal screening examination. The initial screening was based on the GA at birth; 6 weeks after delivery for a GA of fewer than 26 weeks and four weeks after birth for a GA of greater than 26 weeks. The "International Classification of Retinopathy of Prematurity" was used to record the retinal findings. The follow-up schedule was based on the initial result with the 1-3 week interval suggested by ophthalmologists. The indications of treatment for ROP were threshold disease as defined by the CRYO-ROP study and type 1 pre-threshold ROP as determined by the ET-ROP study. Each patient's parent signed an informed consent form before the administration of the intravitreal injection of bevacizumab (Avastin, Genentech Inc., South San Francisco, CA). The off-label use of bevacizumab for ROP was clearly explained.

The medical records of the infants were reviewed to collect prenatal and postnatal variables. Prenatal variables included antenatal steroids, maternal parity, maternal fever, maternal chorioamnionitis, preeclampsia, antepartum hemorrhage, placenta previa and Preterm Premature Rupture of the Membranes (PPROMs). Intrapartum and labor variables included the mode of delivery, GA, BBW, first- and fifth-min Apgar scores and intubation and resuscitation in the delivery room. Postnatal factors including the use of inotropes, respiratory distress syndrome, pneumothorax, sepsis, patent ductus arteriosus and treatment for patent ductus arteriosus. Clinical outcomes, such as mortality, periventricular leukomalacia, and post-hemorrhagic hydrocephalus were analyzed.

In our study, antenatal steroid defined as at least one dose of either betamethasone or dexamethasone is given prenatally. Maternal fever stated as an ear temperature >38°C within 72 hours before delivery. Chorioamnionitis was diagnosed clinically by an obstetrician and recorded in the maternal records with at least one of the following: maternal fever >38°C, maternal leukocytosis, uterus tenderness, foul-smelling amniotic fluid and positive amniotic fluid culture. The use of inotropes defined as either the use of dopamine, dobutamine, milrinone or epinephrine within the first three days of life. Respiratory distress syndrome was diagnosed by the requirement of a mechanical ventilator or surfactant together with the radiographic findings. Pneumothorax was diagnosed by a chest X-ray that may require a thoracocentesis or chest tube insertion. Sepsis was defined if the clinical symptoms occurred within the first three days of life with a positive blood culture. Periventricular leukomalacia signified as parenchymal echo densities/lucencies around the ventricles, documented using cranial ultrasound scans after 21 days of life. Post-hemorrhagic hydrocephalus was diagnosed using a cranial ultrasound or computed tomography with evidence of ventricular dilatation.

Measurement of Serum BDNF and BDNF mRNA

A blood sample was collected aseptically after BSID II was

performed. Blood samples were collected into tubes containing heparin as an anticoagulant. The mononuclear cell was extracted from the blood using the Lymphoprep™, and RNA was extracted following the manufacturer's instructions.

Briefly, RNA was extracted using Blood/Cultured Cell Total RNA Purification Mini Kit (Favorgen, Taiwan) and treated with RNase/DNase Terminator (Protech, Taipei, Taiwan (R.O.C.)) to remove RNA contamination, and 1μg was reverse transcribed (SuperScript II RNaseH-reverse transcriptase; Invitrogen, San Diego, CA, USA) with random primers (Invitrogen) in a total volume of 20 μl. Control reverse transcriptase reactions were performed by omitting the reverse transcriptase enzyme, and PCR was amplified to ensure that the DNA did not contaminate the RNA. Two-step quantitative real-time PCR was conducted using Maxima SYBR green /ROX qPCR Master Mix reagents (Thermo scientific, USA) according to the manufacturer's protocol on a LightCycler 480 real-time PCR system (Roche Diagnostics Ltd., Taipei, Taiwan). An endogenous housekeeping gene, 18S was used. All samples were run in duplicate (2 μl of cDNA/well in a 96-well format). For the relative quantification of gene expression, the comparative threshold cycle (CT) method was employed. The averaged CT was subtracted from the means of endogenous housekeeping genes for each sample, resulting in ΔCT. ΔΔCT was obtained by subtracting the average control ΔCT value from the average experimental ΔCT. The fold increase was established by calculating $2^{-\Delta\Delta CT}$ for experimental vs. control samples.

Developmental Outcomes

A Pediatric psychologist who did not involve in the craniosonographic study was recruited in the developmental assessment. The BSID II provided relevant information to aid in the diagnosis of developmental delay. There are two parameters within BSID II: The Mental Developmental Index (MDI) and the Psychomotor Developmental Index (PDI). The mental index assesses cognition, language, and interpersonal social development. The motor index evaluated both fine and gross motor development. A child with significantly delayed development was defined if the score was below 70; while a score between 85 and 114 was deemed to be normal. The developmental outcomes of the infants that were studied in the two groups were compared and contrasted. Group means calculated from the standard deviation of the mean and group differences were analyzed by using the Analysis of Variance (ANOVA).

The differences of continuous data between patients of the two groups were compared by two-sample t-test or Mann- Whitney U test, when appropriate. Categorical data were analyzed through Chi-square or Fisher's exact test. Entities included in the analysis were gestational age, gender, birth weight, Apgar score, and creatinine level were included as potential covariates, and those that differ statistically were involved in the univariate analysis in a stepwise logistic regression model. A P value of < 0.05 was considered to be significant. Statistical software Minitab 16 was used for analysis.

Results

During the study period, 21 preterm infants received either Avastin or cryotherapy as ROP treatment were included in this study. Table 1 summarizes the demographic characteristics at admission of both groups. The Avastin group had a mean gestational age of 29.2±0.2 weeks and a mean birth body weight of 1181.6±32.3grams, which were not significantly different from that of preterms in the cryotherapy group (28.7±0.3, $p = 0.30$; 1073.2±52.3grams, $p = 0.49$). The demographic characteristics of perinatal and intrapartum factors at admission were not significantly different between the groups.

Factors	Avastin-Group (n=6)	Cryotherapy Group (n=15)	p value
GA (weeks)	29.2±0.20	28.7±0.30	0.46
BBW (gram)	1181.60±32.30	1073.20±52.30	0.26
Apgar-1	5.3±0.30	5.7±0.40	0.39
Apgar-5	7.5±0.20	7.7±0.30	0.11
Antenatal steroid (n)	5	12	1
Chorioamnionitis	0	1	-
PPROM	1	2	-
Pre-eclampsia	0	1	-
GDM	2	1	0.18
Smoking	0	0	-
Hypertension	0	0	-
Gender (f/m)	5-Jan	9-Jun	0.61

GA= Gestational Age, BBW: birth body weight; PPROM = Preterm Premature Rupture of Membrane; GDM= Gestational Diabetes Mellitus was defined as any degree of glucose intolerance with onset or first recognition during pregnancy; Maternal hypertension was defined according to the Working Group (2000) criteria as high blood pressure≥140/90 mmHg after the 20th week of gestation

Table 1: Prenatal and Intrapartum factors of groups of premature infants received either Avastin or cryptherapy.

Table 2 compares the Bayley Scales of Infant Development II between groups of premature infants who received either Avastin or cryotherapy. The MDI and PDI scores at 24 months of corrected age were 82.3±2.9 vs. 72.5±6.8 (p=0.213) and 72.6±2.3 vs. 68.4±4.7 (p=0.759) in Avastin group and Cryotherapy group respectively. The Bayley Scales of Infant Development II were not significantly different between the groups, but the Avastin group tended to have the lower MDI scores. When comparing Avastin and cryotherapy group (Table 3), there is a significantly higher ratio of significant mental delayed (MDI < 70) in Avastin group (83% vs. 33%, p = 0.038) at 24 months of corrected age. We also checked the BDNF levels between the two groups (Table 3). Significantly lower BDNF level is noted in Avastin group (43.2±10.4 vs. 93.3±9.8; p= 0.022).

	Avastin group(n=6)	Cryotherapy group(n=15)	p value
MDI	72.5±6.8	82.3±2.9	0.213
PDI	68.4±4.7	72.6±2.3	0.759
Disability:			
Cerebral palsy (%)	0	1	-
Blindness (%)	0	0	-
Hearing loss (%)	0	0	-
MDI= Mental Developmental Index; PDI = Psychomotor Developmental Index			

Table 2: The Bayley Scales of Infant Development II between groups of premature infants received ether Avastin or cryotherapy at 24 months of corrected age.

	Avastin group (n=6)	**Cryotherapy group (n=15)**	**p value**
Ratio of significant mental delayed	83%(n=5)	33%(n=5)	0.038
BDNF level	43.2±10.4	93.3±9.8	0.022
Significant mental delayed: mental developmental index < 70, BDNF: Brain Derived Neurotrophic Factor.			

Table 3: The ratio of significant mental delayed and BDNF levels between groups of premature infants received either Avastin or cryotherapy at 24 months of corrected age.

Table 4 summarizes the parameters related to development, including periventricular echogenicities persistent greater than two weeks, chronic lung disease, patent ductus arteriosus and necrotizing enterocolitis with Bell stage ≧2a of both groups. There was no significant difference found in any variables between the groups.

	Avastin-Group (n=6)	**Cryotherapy Group (n=15)**	**p value**
PVL (%)	33	20	0.22
CLD (%)	33	46	0.79
PDA (%)	50	40	0.48
NEC (%)	10	7	0.82
PVL= Periventricular Leukomalacia; CLD = Chronic Lung Disease; PDA = Patent Ductus Arteriosus; NEC = Necrotizing Enterocolitis			

Table 4: Parameters Related to Development in groups of premature infants received either Avastin or cryptherapy.

Discussion

There were only a few published studies concerned about the leakage of bevacizumab into the systemic circulation after intravitreal injection for ROP treatment. The long-term safety including the neurodevelopment outcome of intravitreal bevacizumab therapy is unknown, and there is no study investigating the serum BDNF level after Avastin treatment. An animal study has shown that reduced the level of serum BDNF may be associated with neuro-degeneration [18].

In our study, the ratio of significant mental delayed at corrected age 24-month-old who received intravitreal Avastin treatment is significantly higher than the cryotherapy group. Besides, the serum BDNF level checked at corrected age 24-month-old revealed markedly lower in Avastin group than the Cryotherapy group. Although the ocular outcomes significantly improved after intravitreal Avastin injection, the neurodevelopment outcome with the significant mental delayed should be concerned based on our study results. Concerning the neurodevelopment outcome after intravitreal Avastin treatment for ROP, Morin et al. found that there are higher odds of severe neurodevelopmental disabilities in infants who treated with intravitreal bevacizumab versus laser therapy [19]. The finding is compatible with our study result.

Falavarjani and Nguyen reviewed the systemic safety associated with intravitreal injection of anti-VEGF agents [20]. The detectable levels in the systemic circulation of all the intravitreal anti-VEGF agents attribute to suppress the systemic VEGF levels significantly. However, there was limited data regarding the systemic adverse events of intravitreal Avastin. One prospective case series enrolled eight patients investigate the serum VEGF levels for two months after intravitreous injections of bevacizumab, and the resulted showed the plasma VEGF levels were suppressed [21]. The leakage of bevacizumab into systemic circulation should be considered.

Diether and Peter suggest that VEGF may have the role in a neurovascular interface and reduced levels of VEGF implicated

neuron degeneration [22]. From our study, it is prudent to think that a possible leakage of Avastin may decrease serum BDNF level and influence the brain neurovascularization or neuron proliferation. Besides VEGF, BDNF is emerging as potential markers of neurodevelopment because of their neurotrophic effects. Both BDNF and VEGF actively promote younger rats neurite growth [23]. In a series of psychiatric study, serum BDNF levels can quantify the improvement of psychotic symptoms [24-28]. Furthermore, one prospective measured serial serum BDNF concentrations of premature infants after birth and found that the BDNF concentration had a strong correlation with postnatal outcomes that influence neurodevelopment [29]; our study, again supports this idea of taking BDNF as a biomarker for long-term neurodevelopment of premature received Avastin ROP treatment.

Conclusion

From our cohort study, we found that intravitreal Avastin for ROP treatment in premature infants may be related to significantly mental delayed and may have an association with lower BDNF level than conventional cryotherapy treatment at 24 months of corrected age. Further long-term follow-up and multicenter, randomized controlled trials will be needed to assess the neurodevelopment outcome of intravitreal Avastin treatment for ROP.

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Conflict of Interest: None.

References

1. Choi MY, Park IK, Yu YS (2000) Long term refractive outcome in eyes of preterm infants with and without retinopathy of prematurity: Comparison of keratometric value, axial length, anterior chamber depth, and lens thickness. Br J Ophthalmol 84: 138-143.
2. Knight-Nanan DM, Algawi K, Bowell R, O'Keefe M (1996) Advanced cicatricial retinopathy of prematurity-outcome and complications. Br J Ophthalmol 80: 343-345.
3. Gilbert C (2008) Retinopathy of prematurity: A global perspective of the epidemics, population of babies at risk and implications for control. Early Human Development 84: 77-82.
4. Kaiser RS, Trese MT, Williams GA, Cox MS Jr (2001) Adult retinopathy of prematurity: Outcomes of rhegmatogenous retinal detachments and retinal tears. Ophthalmology 108: 1647-1653.
5. Blencowe H, Lawn JE, Vazquez T, Fielder A, Gilbert C, et al. (2013) Preterm-associated visual impairment and estimates of retinopathy of prematurity at regional and global levels for 2010. Pediatric Research 74: 35-49.
6. Kim TI, Sohn J, Pi Sy, Yoon YH (2004) Postnatal risk factors of retinopathy of prematurity. Paediatric and perinatal Epidemiology. 18: 130-134.
7. McLeod DS, Brownstein R, Lutty GA (1996) Vaso-obliteration in the canine model of oxygen-induced retinopathy. Invest Ophthalmol Vis Sci. 37: 300-311.
8. Lange C, Ehlken C, Stahl A, Martin G, Hansen L, Agostini HT (2009) Kinetics of retinal Vado-obliteration and neovascularisation in the oxygen-induced retinopathy (OIR) mouse model. Greece's ARch coin Exp Ophthalmol. 247: 1205-1211.
9. Grunewald M, Abraham I, Dor Y, Bachar-Lustig E, Itin A, et al. (2006) VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells. Cell 124: 175-189.
10. Azad R, Chandra P (2007) Intravitreal bevacizumab in aggressive posterior retinopathy of prematurity. Indian J Ophthalmol 55: 319.
11. Chao MV, Bothwell M (2002) Neurotrophins: to cleave or not to cleave. Neuron 33: 9-12.
12. Haapasalo A, Sipola I, Larsson K, Åkerman KEO, Stoilov P, et al. (2002) Regulation of TRKB surface expression by brain-derived neurotrophic factor and truncated TRKB isoforms. J Bio Chem 277: 43160-43167.
13. Yacoubian TA, Lo DC (2000) Truncated and full-length TrkB receptors regulate distinct modes of dendritic growth. Nat. Neuroscience 3: 342-349.
14. Luikart BW, et al. (2003) In Vick role of truncated tribute receptors during sensory ganglion neurogenesis. Neuroscience 117: 847-858.
15. Huang EJ, Reichardt LF (2001) Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci 24: 677-736.
16. Autry AE, Monteggia LM (2012) Brain-derived neurotrophic factor and neuropsychiatric disorders. Pharmacol Rev 64: 238-258.
17. Ghassabian A, Sundaram R, Chahal N, McLain AC, Bell E, et al. (2017) Determinants of neonatal brain-derived neurotrophic factor and association with child development. Dev Psychopathol 29: 1499-1511.
18. Ola MS, Nawaz MI, El-Asrar AA, Abouammoh M, Alhomida AS (2013) Reduced levels of brain derived neurotrophic factor (BDNF) in the serum of diabetic retinopathy patients and in the retina of diabetic rats. Cell Mol Neurobiol 33: 359-367.
19. Morin J, Luu TM, Superstein R, Ospina LH, Lefebvre F, et al. (2016) Neurodevelopmental Outcomes Following Bevacizumab Injections for Retinopathy of Prematurity. Pediatrics 137: e 20153218-27.
20. Falavarjani KG, Nguyen QD (2013) Adverse events and complications associated with intravitreal injection of anti-VEGF agents: a review of literature. Eye (Lond) 27: 787-794.
21. Wu WC, Lien R, Liao PJ, Wang NK, Chen YP, et al. (2015) Serum levels of vascular endothelial growth factor and related factors after intravitreous bevacizumab injection for retinopathy of prematurity. JAMA Ophthalmol 133: 391-397.
22. Lambrechts D, Carmeliet P (2006) VEGF at the neurovascular interface: therapeutic implications for motor neuron disease. Biochim Biophys Acta 1762: 1109-1121.
23. Lin G, Shindel AW, Fandel TM, Bella AJ, Lin CS, et al. (2010) Neurotrophic effects of brain-derived neurotrophic factor and vascular endothelial growth factor in major pelvic ganglia of young and aged rats. BJU Int 105: 114-120.
24. Murphy BP, Pang TY, Hannan AJ, Proffitt TM, McConchie M, et al.

(2014) Vascular endothelial growth factor and brain-derived neurotrophic factor in quetiapine treated first-episode psychosis. Schizophr Res Treatment: 719395.

25. de Azua SR, Matute C, Stertz L, Mosquera F, Palomino A, et al. (2013) Plasma brain-derived neurotrophic factor levels, learning capacity and cognition in patients with first episode psychosis. BMC Psychiatry 13: 27-36.

26. Toyooka K, Asama K, Watanabe Y, Muratake T, Takahashi M, et al. (2002) Decreased levels of brain-derived neurotrophic factor in serum of chronic schizophrenic patients. Psychiatry Res 110: 249-257.

27. Tan YL, Zhou DF, Cao LY, Zou YZ, Zhang XY (2005) Decreased BDNF in serum of patients with chronic schizophrenia on long-term treatment with antipsychotics. Neurosci Lett 382: 27-32.

28. Grillo RW, Ottoni GL, Leke R, Souza DO, Portela LV, et al. (2007) Reduced serum BDNF levels in schizophrenic patients on clozapine or typical antipsychotics. J Psychiatr Res 41: 31-35.

29. Rao R, Mashburn CB, Mao J, Wadhwa N, Smith GM, et al. (2009) Brain-derived neurotrophic factor in infants <32 weeks gestational age: correlation with antenatal factors and postnatal outcomes. Pediatr Res 65: 548-552.