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Effects of Intravitreal Anti-VEGF Therapy on the Clinical Course of Bronchopulmonary Dysplasia

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Abstract

Objectives: To demonstrate the effects of the injection of intravitreal bevacizumab (IVB) on the clinical course of established bronchopulmonary dysplasia (BPD).
Methods: This is a multicenter retrospective case-control study performed without any randomization or masking procedure. A total of 70 patients with BPD, including 35 cases (the IVB group) and 35 controls (the control group) were studied. Patients in the IVB group received intravitreal anti-VEGF (bevacizumab) treatment for type 1 prethreshold retinopathy of prematurity (ROP). The control group consisted of infants with BPD whose gestational age, birthweight, and gender were matched with those of the IVB group. None of the infants in the control group needed to be treated for ROP using either anti-VEGF or laser photocoagulation.
Results: There was no statistically significant difference (P = 0.11) between the groups in terms of the total duration of oxygen prior to the IVB injection (65.9 ± 23.5 and 79.1 ± 33.1 days in the IVB group and control group, respectively). However, after the injection of IVB, the total duration of oxygen was significantly lower in the IVB group (mean 7 (1 - 70) days vs. 16 (1 - 98) days, P = 0.01). In 14 cases with mild BPD and their matched controls, the median time (25% - 75%) for the discontinuation of oxygen therapy was 3 (2 - 7) days and 10 (5 - 15) days, respectively (P = 0.36). In 21 cases with moderate and severe BPD and their matched controls, the median time (25 - 75%) for the discontinuation of oxygen therapy was 14 (7 - 21) days and 22 (16 - 43) days, respectively (P = 0.024).
Conclusions: Intravitreal bevacizumab injection treatment for ROP cases with BPD was found to be associated with a shorter duration of oxygen use. The results of the study not only demonstrate a pathogenic correlation between ROP and BPD through an abnormal vasculogenesis, but also raise a question regarding whether or not the systemic side effects of IVB are actually adverse.

Keywords: Bronchopulmonary Dysplasia, Retinopathy of prematurity, Bevacizumab

1. Background

The most common pulmonary disease following preterm birth and neonatal intensive care is chronic lung disease of prematurity, also known as bronchopulmonary dysplasia (BPD) (1), which has consequences for the later lung function of affected patients (2).

BPD was first reported to be lung damage resulting from barotrauma and volutrauma following prolonged mechanical ventilation (MV). The histological features of MV-related BPD include alternating areas of atelectasis and hyperinflation, severe airway epithelial lesions (hyperplasia and squamous metaplasia), airway smooth muscle hyperplasia, and extensive fibroproliferation (3). However, even in the absence of MV, premature birth interrupts normal intrauterine lung development, and it impairs pulmonary function during the first years of life and beyond independently of further neonatal disease (4-6). The so-called "new BPD" is characterized by an arrest in lung maturation, dimensional growth, and alveolar septation, which leads to simplified alveolar structures, dysmorphic capillary configuration, and variable interstitial cellularity (7).

On the other hand, BPD and retinopathy of prematurity (ROP) are two common morbidities that have always been said to exhibit pathogenic interaction or correlation. A National Institute of Child Health and Development (NICHD) Neonatal Research Network trial (8), as well as a study by Vento et al. (9), previously demonstrated a possible pathogenic interaction between ROP and BPD, at least by means of oxygen exposure.

In addition, in recent years a new treatment modality involving the intravitreal injection of anti-VEGF (vascular endothelial growth factor) agents (intravitreal bevacizumab (IVB)) has been used in the treatment of ROP with high success rates of up to 100% in even the most aggressive forms of the disease (10-14). However, anti-VEGF agents can gain access to the systemic circulation following an intravitreal injection (15-19), and the systemic adverse effects of intravitreal anti-VEGF agents have always been a concern. VEGF is a mediator associated with vasculogene-

sis and angiogenesis, and it induces vascular permeability in addition to endothelial cell proliferation and migration (20). Intravitreal anti-VEGF agents have been reported to be associated with detectable levels in the systemic circulation that may significantly suppress systemic VEGF levels (21). There is also a scientific rationale for the potential occurrence of effects on the clinical course of BPD.

Nevertheless, we have previously reported that in terms of possible systemic side effects, there was an improvement in the clinical course of BPD following the injection of IVB (i.e., a decrease in oxygen dependency, more rapid advancement to oral nutrition, and weight gain) (10).

2. Objectives

We designed this study to demonstrate the effects of the injection of intravitreal bevacizumab on the clinical course of established BPD.

3. Methods

This multicenter retrospective case-control study was performed without any randomization or masking procedure. The study was approved by the ethics committee of Cerrahpasa School of Medicine. Two centers contributed to the study, namely Cerrahpasa School of Medicine and the Zeynep Kamil Women and Children's Training and Research Hospital, Istanbul. An informed consent form was signed by the parents of all patients in the study group prior to the IVB injection.

A total of 70 patients with a diagnosis of BPD, including 35 cases (the IVB group) and 35 controls (the control group), were recruited. The IVB group consisted of patients with BPD who had received intravitreal anti-VEGF (bevacizumab) treatment (IVB) for type 1 prethreshold ROP. To create the control group, infants with similar gestational age, birthweight, and sex who were born immediately before or after the patients in the study group and who were diagnosed with BPD were identified from the institutional database. None of the infants in the control group needed either anti-VEGF or laser photocoagulation (LPC) treatment for ROP. All demographic and clinical data were retrieved from the patients' files.

Bronchopulmonary dysplasia was defined as the need for oxygen treatment at the 28th postnatal day. It was classified as mild BPD if no oxygen was needed at the 36th postconceptional week; moderate if the oxygen need was less than 30% at the 36th postconceptional week; and severe if the oxygen need was more than 30% at the 36th postconceptional week (1).

The oxygen need in terms of total duration (days) and duration (days) after IVB application was compared between the groups. The oxygen need in the control group was calculated from the day of IVB treatment in the matched case. In order to evaluate any similarities and/or changes in the systemic status of the groups, any associated co-morbidities and clinical features such as patent ductus arteriosus (PDA), necrotizing enterocolitis (NEC), intraventricular hemorrhage, postnatal steroid usage, and duration of hospitalization were also compared.

Intravitreal bevacizumab injection procedure: The intravitreal bevacizumab injection procedures were performed according to Dr. Huseyin Yetik's intravitreal injection technique under indirect ophthalmoscopic visualization and illumination. After the eyes were prepared with 5% povidone-iodine in the standard fashion, and while visualizing the tip of the needle through the dilated pupil under binocular indirect ophthalmoscopic visualization and illumination, 0.625 mg (0.025 mL) of bevacizumab (Altuzan 100 mg/4 ml flacon, Roche, Turkey) was injected using a 30-G needle into the vitreous cavity approximately 1 mm behind the limbus via the pars plicata under topical anesthesia. An experienced nurse helped to secure the infant during the procedure. All injections were performed by the same surgeon (Dr. H.Y.). After the injections, retinal artery patency was checked. Topical antibiotic drugs were then administered for five days (see supplemental video S2 of (10)).

For the statistical analyses, the NCSS (number Cruncher statistical system) 2007 and PASS (power analysis and sample size) 2008 statistical software (Utah, USA) programs were used. The Mann-Whitney U test was used to assess the continuous nonparametric variables, while the t-test was used for the parametric variables. Statistical significance was assessed as $P < 0.05$.

4. Results

In the IVB group, 14 mild, 15 moderate, and six severe cases of BPD were observed. In order to achieve maximal similarity between the groups, the same number of cases for each severity subgroup was retrospectively selected for enrollment in the control group. Anti-VEGF therapy was administered at a median of 34 $\pm$ 2.1 postconceptional weeks (min-max=31 - 39 weeks).

There was no statistically significant difference ($P = 0.11$) between the two groups in terms of total oxygen duration prior to IVB injection (65.9 $\pm$ 23.5 and 79.1 $\pm$ 33.1 days in the IVB group and control group, respectively). However, following IVB application the total oxygen duration was significantly lower in the IVB group (mean 7 (1 - 70) days vs. 16 (1 - 98) days, $P = 0.01$) (Table 1). In 14 cases of mild BPD and their matched controls, the median time (25 - 75%) for the discontinuation of oxygen therapy was 3 (2 - 7) days and

10 (5 - 15) days, respectively (P = 0.36). In 21 cases with moderate and severe BPD and their matched controls, the median time (25 - 75%) for the discontinuation of oxygen therapy was 14 (7 - 21) days and 22 (16 - 43) days, respectively (P = 0.024).

In terms of the comparison between associated co-morbidities such as patent ductus arteriosus, necrotizing enterocolitis, intraventricular hemorrhage, postnatal steroid usage, and duration of hospitalization, as well as the duration of oxygen use and mechanical ventilation, there were no statistically significant differences between the groups (Table 1). As a surrogate for postnatal growth, there was no difference between the two groups' discharge weights (2451 g vs. 2601 g). Steroid use was similar in both groups (Table 1).

5. Discussion

Since the comparison of the associated co-morbidities and clinical features showed no significant differences between the groups prior to the injection of IVB, this study was primarily focused on the clinical course of BPD.

Bronchopulmonary dysplasia and ROP are developmental diseases affecting preterm infants, both of which represent a disruption or arrest to the maturation process of the associated tissues or organs. There have been several studies that emphasize the importance of VEGF in the alveolar development of these tiny infants. Jakkula et al. showed that the inhibition of angiogenesis simultaneously decreases the number of alveoli in developing rat lungs (22). In another study, the VEGF levels in the tracheal aspirate samples of BPD patients were found to be decreased (23). This might indicate a possible role of low levels of VEGF in the pathogenesis of BPD. Brown et al. showed that VEGF acts directly on the pulmonary epithelium and stimulates alveolarization (24). Furthermore, Maniscalco et al. studied preterm rats and found that VEGF signal disruption due to mechanical ventilation may have an impact on the development of BPD (25).

In contrast to these well-known studies, our study demonstrated a better clinical course of established BPD after intravitreal anti-VEGF bevacizumab injection. Despite the absence of any statistically significant difference between the groups in terms of the total duration of oxygen usage prior to IVB injection, the total oxygen duration was significantly less in the IVB group (mean 7 (1 - 70) days vs. 16 (1 - 98) days, P = 0.01) after IVB injection in the present study (Table 1). Furthermore, this statistical significance became more prominent if the severity of the BPD worsened, since in mild BPD cases and their matched controls, the median time (25% - 75%) for the discontinuation of oxygen therapy was 3 (2 - 7) days and 10 (5 - 15) days, respectively, and the difference was insignificant (P = 0.36). However, for moderate and severe BPD cases and their matched controls, the median time (25 - 75%) for the discontinuation of oxygen therapy was 14 (7 - 21) days and 22 (16 - 43) days, respectively, and the difference was more significant (P = 0.024). Therefore, a very important question remains to be answered: Aside from the decrease in VEGF levels, how can this better clinical course of BPD be explained?

VEGF plays a central role in the life and death of pulmonary vascular endothelial cells, and the main sites of VEGF production are type II pneumocytes and activated alveolar macrophages (26). Nevertheless, strict control of VEGF expression is necessary during alveolar development. Le Cras et al. demonstrated in VEGF transgenic mice that increased VEGF levels cause a six-fold increase in the bronchoalveolar lavage fluid (BALF) protein levels and pulmonary hemorrhage in neonates. Furthermore, half the VEGF transgenic mice died prior to reaching two weeks of age, most likely due to pulmonary hemorrhage. The lungs of VEGF transgenic mice with respiratory distress exhibited gross blood and VEGF overexpression, which was shown to result in increased mortality, pulmonary hemorrhage, hemosiderosis, alveolar remodeling, and inflammation (27). Therefore, similar to the pathogenesis of ROP, the overexpression of VEGF could have played a key pathogenic role in the normal development of the lungs in cases of BPD in the same way it does in the normal development of retina in cases of ROP.

On the other hand, postnatal intratracheal adenovirus-mediated VEGF gene therapy improved survival, promoted lung capillary formation, and preserved alveolar development in neonatal rats exposed to hyperoxia (28). As stated in the study, the mechanisms and signal transduction pathways that regulate normal alveolar development remain poorly understood, and even less is known about how these pathways are altered in disease. The interactions between the airways and blood vessels are critical for normal lung development, suggesting that a coordinated and timely release of vascular-specific growth factors from respiratory epithelial cells promotes alveolar development (28).

In particular, the coordinated and timely release of vascular-specific growth factors noticed in this study should be very important for almost all morbidities associated with prematurity. According to the biphasic theory of ROP, in order to induce normal retinal vascularization, the expression of VEGF increases during the first phase; however, it continues to feed and increase pathological vascularization during the second phase. In other words, in the first phase, VEGF seems useful for inducing normal vascularization, while in the second phase, when normal vas-

Table 1. Clinical Features and Outcomes of the Patients

	IVB Group (n = 35)	Control Group (n = 35)	P
Gestational week	26.6 ± 1.6	26.6 ± 1.5	0.90
Male/female	18/17	16/19	0.63
Birthweight (g)	902.1 ± 262	879 ± 242	0.82
Patent ductus arteriosus	14/35	18/35	0.33
Necrotizing enterocolitis	6/35	2/35	0.13
Intraventricular hemorrhage	14/35	8/35	0.30
Invasive ventilation (days)	14 (1 - 70)	11 (1 - 54)	0.13
Non-invasive ventilation (days)	21 (1 - 70)	20 (3 - 67)	0.40
Postnatal steroids	14/35 (39.1%)	9/35 (60.9%)	0.20
Duration of hospitalization (days)	82.7 ± 22.9	95.8 ± 31.5	0.10
Bodyweight at discharge (g)	2451 ± 586	2606 ± 570	0.26
Duration of oxygen use (days)	65.9 ± 23,5	79.1 ± 33.1	0.11
Duration of oxygen use after IVB (days)	7 (1 - 70)	16 (1 - 98)	0.01

cularization cannot be achieved, the presence of VEGF becomes harmful. Therefore, the results following the increase or blockage of VEGF should be totally dependent on the phase of the disease. Thus, even though VEGF-induced angiogenesis is also partly mediated by nitric oxide and the treatment of hyperoxia-exposed rats with an NO donor has increased both the VEGF mRNA and protein levels and restored the expression level of the key controllers of alveolarization (29), inhaled NO in human preterm neonates has not improved survival without BPD (30). In addition, in the study by Le Cras et al. it was shown that although chronic increases in VEGF did not alter postnatal lung morphogenesis, vascular leakage and pulmonary hemorrhage were observed in VEGF transgenic mice, resulting in a 50% increase in neonatal mortality (27). In addition to alveolar hemorrhage, evidence of inflammation, air space remodeling, and pulmonary hemosiderosis was observed, and the VEGF levels caused a six-fold increase in the protein levels and pulmonary hemorrhage in neonates. Previous studies have shown decreased VEGF levels in the lungs of infants who died of BPD. On the basis of these findings, postnatal intratracheal adenovirus-mediated VEGF gene therapy serves to improve survival and preserve alveolar development (28). Elevated VEGF levels have also been reported in sepsis, and this has been thought to lead to the capillary leak syndrome seen in sepsis (31). The increased expression of VEGF has been associated with several respiratory diseases, including bronchitis, airflow limitation, and asthma (32, 33). Thus, in the present study, the statistical significance of the decrease in oxygen dependency increased as the severity of the BPD worsened.

In our study, in both mild BPD cases and moderate to severe BPD cases, the duration of oxygen dependency was significantly lower in patients who received anti-VEGF therapy. Although the amount of oxygen administered decreased in some patients with moderate to severe BPD, we calculated the duration of time for the absolute discontinuation of oxygen therapy to be a better marker of oxygen dependency.

VEGF is mitogenic for endothelial cells, as well as inducing capillary permeability and regulating endothelial cell migration and tube formation (34). A histologic study has shown that intravitreal anti-VEGF induces apoptosis and lessens fenestration in vascular endothelial cells, indicating that the treatment affects vascular endothelial cells (35). In another study, the intravitreal injection of anti-VEGF was shown to inhibit leukocyte trafficking in the retina, which suggests that anti-VEGF therapy could serve as a treatment for retinal inflammation (36).

Intravitreal anti-VEGF therapy has been increasingly used in severe ROP cases; hence, whether or not the systemic leakage of anti-VEGF can cause any short- or long-term adverse effects on the tissues or organs has always been an issue (10-14). The effects of this therapy on other tissues, including the lungs, were not investigated thoroughly in the present study, although we have previously reported a better systemic clinical course of BPD in preterm babies after the injection of IVB (10). VEGF may have protective and regenerative effects in instances of lung injury, but it also contributes to non-cardiogenic pulmonary edema by increasing vascular permeability. Therefore, the down-regulation of VEGF observed in instances

of acute lung injury is thought to represent a protective mechanism aimed at limiting endothelial permeability (37). This may occur at the expense of a decrease in the number of capillaries. Similar mechanisms may be effective in the development of other diseases associated with aberrant vasculogenesis or epithelial morphogenesis, for example, necrotizing enterocolitis (10).

In summary, the results of our study suggest that anti-VEGF treatment for premature infants may ameliorate the oxygen dependence of these infants. The results of this study are very important in terms of both decreasing apprehension regarding the systemic adverse effects of anti-VEGF injections and having the potential to promote the systemic anti-VEGF treatment of almost all morbidities of prematurity (i.e., ROP, BPD, NEC, and intracranial complications) simultaneously in the near future. However, further studies are needed to demonstrate and reproduce the results of this study.

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