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Efficacy of azithromycin for prevention of bronchopulmonary dysplasia (BPD)

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Aim: Bronchopulmonary dysplasia (BPD) remains one of the most serious and challenging complications in premature infants. This study was conducted to evaluate the efficacy of azithromycin in the prevention of BPD in very low birth weight preterm infants

Materials and methods: Preterm neonates with birth weight less than 1500 g were enrolled in a prospective randomized controlled clinical trial. One hundred eight neonates were randomly allocated to the intervention group (n = 56) or control group (n = 52). The intervention group received oral azithromycin 10 mg/kg for 1 week followed by 5 mg/kg for another week. The outcome measures were development of BPD at 28 days of birth and 36 weeks postmenstrual age (PMA).

Results: The mean gestational age of the studied patients was 29.2 ± 3.6 weeks and their birth weight was 1173.9 ± 261 g. There was no significant difference in participants' sex distribution or their gestational age between the 2 groups. Twenty-one (43%) of the 52 patients in the control group met the definition of BPD at 28 days while 14 (25%) did in the azithromycin group (P = 0.04). Twelve patients were oxygen dependent (moderate to severe BPD) at 36 weeks PMA; 9 of them were from the control group (P = 0.04).

Conclusion: Our study suggests that azithromycin is effective in reducing the incidence of BPD in very low birth weight infants. More studies are needed with large numbers of patients before recommending routine use of this relatively safe drug for prevention of BPD.

Key words: Bronchopulmonary dysplasia, very low birth weight infants, prematurity, azithromycin, prevention

Introduction

Bronchopulmonary dysplasia (BPD) was initially described by Northway in 1967 as an evolving radiologic pattern of lung injury among premature infants who are mechanically ventilated and receiving supplemental oxygen (1). Currently, this severe form of chronic lung disease is less common and has been replaced by a milder form of the disease. This new form of BPD is associated with a disruption of lung organogenesis and impaired pulmonary function during the first years of life (2,3). This milder form of lung damage occurs in infants who initially have only mild pulmonary disease and do not require high airway pressures or FiO_2 and demonstrate a different radiologic picture that shows diffuse haziness and a fine lacy pattern (4). The incidence of BPD in premature infants with respiratory distress syndrome (RDS) who receive mechanical ventilation is inversely related to gestational age and birth weight (5). Mechanical ventilation with positive pressure and prematurity have been considered the most important factors in the pathogenesis of BPD; other factors that can contribute to the pathogenesis of BPD

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are oxygen toxicity, pulmonary or systemic infections, pulmonary vascular damage, and pulmonary edema induced by patent ductus arteriosus or excessive fluid administration (6-10). There is increasing evidence for the role of antenatal and postnatal infections in the development of BPD. Several studies suggested an association between *Ureaplasma urealyticum* tracheal colonization and the development of severe respiratory failure and BPD in very low birth weight infants (11-14).

When considering *Ureaplasma urealyticum* as a cause of BPD, it would be reasonable to expect prevention of BPD by antibiotic treatment against *Ureaplasma*. Macrolide antibiotics have anti-inflammatory actions that include inhibiting pro-inflammatory cytokines (such as IL-1, IL-6, and TNF-α), inhibiting inflammatory transcription factors (NF-KB), acting as free radical scavengers, inhibiting neutrophil chemotaxis, and inhibiting superoxide generation. Azithromycin is a macrolide that modulates neutrophil function, and reduces chemokine and interleukin-6 (15). We conducted a clinical trial to evaluate the efficacy of azithromycin in the prevention of BPD in very low birth weight preterm infants.

Materials and methods

This was a randomized controlled clinical trial carried out at the neonatal intensive care (NICU) unit of Al-Zahra teaching hospital, Tabriz, Iran, a university level III neonatal center in the northwest of Iran, from April 2010 to December 2010. Consecutive preterm newborn infants of gestational age less than 32 weeks and birth weight less than 1500 g that were admitted to the NICU were eligible for enrollment into our study. Infants with congenital malformations, known syndromes, or chromosomal anomalies and infants with signs compatible with the diagnosis of necrotizing enterocolitis (NEC) in the first week of life were excluded from this study. Tabriz University of Medical Sciences ethics committee approved the study. Informed parental consent was obtained when the infant met the inclusion criteria. Gestational age was determined by the last menstrual period and first or second trimester ultrasonography when available. Accuracy of gestational age determination was confirmed by Ballard assessment (16). After birth and admission to the NICU, all infants were continuously evaluated for the presence of respiratory distress syndrome and need for supplemental oxygen. Infants with respiratory distress and grunting were initially placed on continuous positive airway pressure (CPAP) of 5-7 cm H_2O. Premature infants who needed a fraction of inspired oxygen (FiO_2) more than 40% to maintain adequate oxygenation and radiologic findings compatible with RDS were intubated for surfactant administration with the INSURE method. Patients were randomly allocated into 2 groups. Randomization was performed 1:1 using a computer-generated randomization list prepared by an independent statistician not involved in the rest of the investigation. Based on the list, sequentially numbered sealed opaque envelopes containing cards with group assignment were prepared. The intervention group received oral azithromycin (Zithromax, Pfizer, Italy) 10 mg/kg/day initiated from day 7 of birth for 1 week followed by 5 mg/kg/day oral azithromycin for an additional 1 week. The drug was discontinued if the infants were weaned from supplemental oxygen and discharged home before completing the 2-week treatment course. The primary outcomes were development of BPD at 28 days of life and oxygen dependency at 36 weeks postmenstrual age (PMA). Secondary outcomes included length of hospital stay and duration of oxygen dependency. The criteria for diagnosis of BPD were continued dependency on supplemental oxygen at 28 days of life or longer with a compatible chest radiograph. Definition of moderate to severe BPD in preterm neonates with gestation age less than 32 weeks was dependence on supplemental oxygen at 36 weeks PMA (17). The diagnosis of BPD was recorded by 2 independent senior attending neonatologists who did not know the group assignment of the neonates. The respiratory nurses were also blinded to the patient groups. The same respiratory management protocol was used for treatment of studied patients. The fraction of inspired oxygen was adjusted to maintain pulse oximetry saturation between 90% and 92%. Diagnosis of sepsis was based on the presence of clinical signs of sepsis accompanied by positive blood culture. Patent ductus arteriosus (PDA) was diagnosed based on clinical signs confirmed by echocardiography done by an expert pediatric cardiologist. Ibuprofen 10 mg/

kg followed by 5 mg/kg/day for 2 consecutive days were used to treat echographic hemodynamically significant PDA. Liver function tests were performed at 21-28 days of life in patients of both groups. Hearing screening was done at a follow-up visit.

In calculating the sample size required for this randomized study, it was considered that, in a previous pilot study including very low birth weight premature infants who had BPD in our center, 41% of the patients were satisfied after 1 month. A difference of 30% in the satisfaction rate between the 2 study groups was considered clinically relevant. To have an 80% chance of detecting such a difference at an overall statistical significance level of 5%, 50 patients per group were required. Allowing for dropouts, the aim was to recruit a total of about 128 patients.

Statistical analysis

The demographic characteristics of neonates were compared by using Student's t test, chi-square test, or Fisher's exact test as appropriate. Student's t test or Mann-Whitney rank sum test and chi-square test were used to compare between the 2 study groups. $P < 0.05$ was considered statistically significant. Data were analyzed using Sigma Stat version 3.5 and SPSS version 17.0/Win.

Results

One hundred twenty-eight infants weighing ≤ 1500 g at birth were admitted to the NICU during the study period. Twenty patients who met the weight criteria were excluded from the study: 11 patients died within the first week of life and 2 developed signs compatible with NEC in the first week of life. Two patients had congenital malformations. The parents refused to grant consent for 5 patients. One hundred eight infants were enrolled in the study and randomly categorized into 2 groups. The intervention group consisted of 56 infants and the control group 52 neonates. The mean gestational age of the studied patients was 29.2 ± 3.6 weeks and their birth weight was 1173.9 ± 261 g. The comparison between the 2 studied groups revealed no significant difference in participants' sex distribution or their gestational age and maternal risk factors as shown in Table 1. There was a positive history of preeclampsia in 8 infants who developed BPD ($P = 0.12$). Regarding the need for surfactant replacement therapy, there was no significant difference among patients in the 2 groups [33 neonates (58.9%) in the intervention group vs. 30 neonates (57.7%) in the control group ($P = 0.97$)]. Thirteen infants (23.2%) underwent mechanical ventilation in the intervention group and 11 (21.1%)

Table 1. Comparison of some demographic characteristics of neonates in the intervention and control groups.

Variable \ Group	Intervention (N = 56)	Control (N = 52)	P value
Gestational age (weeks)	29.8 ± 2.5	29.7 ± 2.3	0.93
Birth weight (g)	1177.1 ± 221	1212.9 ± 235.7	0.42
Sex (m/f)	34/22	35/17	0.54
Apgar score			
1 min	5.8 ± 2.3	6.5 ± 1.9	0.09
5 min	8 ± 1.5	8.4 ± 1.4	0.007
PROM	11(%)	6 (%)	0.29
preeclampsia	19 (34%)	14 (27%)	0.27
IUGR	4	3	0.77

PROM = Preterm rupture of membranes
IUGR = intrauterine growth retardation

in the control group (P = 0.49). CPAP was used in 40 infants (73%) in the intervention group and in 39 (75%) in the control group (P = 0.99). Vitamin A was administered to 16 (30.7%) neonates in the control group and to 16 (23.5%) in the azithromycin group (P = 0.08). Treatment with aminophylline was given in 38 (71.1%) and 42 (71.4%) patients in the control and intervention groups, respectively (P = 0.83). Blood culture was positive in 5 neonates; 3 of them were in the azithromycin group (P = 0.7). The use of other antibiotics was positive in 64 patients (36 in the intervention group and 28 in the control group) (P = 0.45).

C-reactive protein (CRP) was measured qualitatively in 105 neonates and it was positive in 10 patients. Nine infants with positive CRP were in intervention group (P = 0.01). Three patients with positive CRP developed BPD (P = 0.86).

Five patients in the intervention group were discharged before the age of 21 days and the duration of treatment with azithromycin was less than 2 weeks. The mean duration of azithromycin therapy in the intervention group was 13.57 ± 1.52 days. The mean duration of hospital stay in the intervention group was 38.4 ± 21 days and in the control group it was 36.1 ± 27.2 days without a significant difference (P = 0.62). Twenty-one (43%) of the 52 patients in the control group met the definition of BPD at 28 days whereas 14 (25%) did in the intervention group (P = 0.04). Twelve patients had BPD at 36 weeks PMA; 9 of them were from the control group (P = 0.04). The mean duration of need for supplemental oxygen was 17.7 ± 2.8 and 23.95 ± 4.2 days in the intervention and control groups, respectively (P = 0.01). The mean FiO_2 needed at 28 days after birth for infants in the intervention and control groups was 23.9 ± 0.7 and 26.2 ± 1.1, respectively (P = 0.02). PDA was diagnosed in 33 infants; 18 of them were in the azithromycin group (P = 0.29). Hemodynamically significant PDA needing ibuprofen was seen in 18 patients; 10 of them developed BPD (P = 0.02). The incidence of intraventricular hemorrhage, NEC, patent ductus arteriosus and abnormal hearing screen was not significantly different between the 2 groups (Table 2). The rate of antenatal steroid treatment to induce lung maturation did not differ significantly between the 2 groups [31 infants (59.6%) in the control group and 34 (60.7%) in the intervention group (P = 0.90)].

Table 2. Comparison the complications of prematurity among patients in the 2 groups.

Complication \ Group		Intervention (N = 56)	Control (N = 52)	P value
IVH				
Grade	I	17 (33.5%)	17 (32.7%)	0.32
	II	-	2 (3.8%)	0.20
	III	-	1 (1.9%)	0.24
	IV	-	-	
NEC		1 (1.8%)	-	
PDA		20 (35.7%)	13 (25%)	0.29
Sepsis		3 (5.3%)	2 (3.8%)	0.99
Vomiting		9 (16.1%)	7 (13.5%)	0.79
Abnormal hearing screen		2 (3.6%)	3 (5.8%)	0.58
SGOT		61.1 ± 13.7	36.6 ± 2.9	0.14
SGPT		15.5 ± 2.4	12.8 ± 2.2	0.43
ALKP		766.9 ± 47	736.8 ± 66.5	0.71

IVH = intraventricular hemorrhage, NEC = necrotizing enterocolitis, PDA = patent ductus arteriosus, ALKP = alkaline phosphates

Discussion

BPD was diagnosed in 25% of infants who received azithromycin and in 43% of infants in the control group (P = 0.04). There are reports that azithromycin causes improved quality of life in patients with cystic fibrosis and bronchiolitis obliterans (18,19). Ballard et al. used intravenous azithromycin for prevention of BPD in a small single center pilot study and showed significant reductions in length of hospital stay, duration of mechanical ventilation, and incidence of treatment with dexamethasone, but no reduction in incidence of BPD (20). In another study they showed a decrease in BPD or death in the azithromycin group who had *Ureaplasma urealyticum* positive aspirates (21). Previous studies have suggested that genital *mycolplasmas* may contribute to occurrence of BPD in a proportion of premature infants based on applying different sampling methods and different diagnostic tests (14,22-24). The effects of colonization or infection with *Ureaplasma urealyticum* on the risk and severity of BPD remain controversial. Available evidence from 2 small controlled studies does not support the use of routine testing for this infection in preterm infants or prophylactic treatment with erythromycin in these high-risk infants (25). *Ureaplasma* was implicated in the development of the pulmonary inflammatory response observed in infants who progressed to develop BPD (26). Knowledge of the biology of *Ureaplasma* species and their behavior in the respiratory tract of preterm neonates suggest that lung disease associated with these organisms is not necessarily due to direct damage from the bacteria themselves, but rather because of their potent stimulation of proinflammatory cytokines or perhaps blockage of counter-regulatory cytokines (14). The pathogenesis of BPD is multifactorial and it is often difficult to determine the effect of one particular risk factor because of overlap between risk factors. We showed that oral azithromycin is effective in reducing chronic oxygen dependency in preterm infants. Although the reduced incidence of BPD in azithromycin group infants in our study may suggest a role of *mycoplasmas* in the pathogenesis of BPD, it may be due to the anti-inflammatory effects of azithromycin. Culic et al. showed that 3-day treatment of healthy human subjects with standard dose azithromycin exerts acute effects on the release of neutrophil granular enzymes, oxidative burst, and oxidative protective mechanisms. Initial degranulation of circulating neutrophils by azithromycin is likely to contribute to its antibacterial effects at sites of infection. However, a more prolonged degranulation of circulatory neutrophil could represent a potential anti-inflammatory effect (15). It is hypothesized that BPD progression results from antenatal inflammatory exposures that are dysregulating postnatal inflammation and lung development (7). We did not find more hepatic side effects or hearing loss in intervention group neonates than in the control group during hospitalization and follow-up. Our patients had received azithromycin for 2 weeks. The exact age of treatment start, appropriate dose, and duration of treatment with azithromycin for prevention of BPD are not defined.

Risk factors of BPD including mechanical ventilation and sepsis had similar frequency among patients with and without BPD in our study. In our study, hemodynamically significant PDA was more common in infants who developed BPD. The lack of a significant difference among patients who developed BPD and patients without BPD with respect to surfactant replacement therapy may be due to the fact that surfactant became widely available and now is the standard care in the management of premature infants with respiratory distress syndrome.

Our study had a limitation in that we did not collect tracheal aspirates for *Ureaplasma urealyticum* culture. Testing for *Ureaplasma* in clinical practice is too expensive and the results are often delayed.

Conclusion

Our study suggests that azithromycin in very low birth weight infants may effectively reduce the incidence of BPD. Further studies are recommended to assess the efficacy of this relatively safe treatment in a large group of patients before any recommendations for its routine use can be made.

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