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Original Articles

Effects of Early Inhaled Beclomethasone Therapy on Tracheal Aspirate Inflammatory Mediators IL-8 and IL-1ra in Ventilated Preterm Infants at Risk for Bronchopulmonary Dysplasia

Gopal K. Gupta, MD,[1] Cynthia H. Cole, MD, MPH,[1]* Soraya Abbasi, MD,[2] Serkalem Demissie, MPH,[3] Charles Njinimbam, MD,[1] Heber C. Nielsen, MD,[1] Theodore Colton, ScD,[3] and Ivan D. Frantz III, MD[1]

Summary. We tested the hypothesis that inhaled beclomethasone therapy for prevention of bronchopulmonary dysplasia (BPD) reduces pulmonary inflammation. As part of a randomized, placebo-controlled trial, interleukin-8 (IL-8) and interleukin-1 receptor antagonist (IL-1ra) concentrations in tracheal aspirates were measured as markers of pulmonary inflammation. On study days 1 (baseline), 8, 15, and day 28 of age, samples were obtained from enrolled infants (birth weights <1,251 g, gestational age <33 week, 3 to 14 days of age) who remained ventilated and had not received systemic glucocorticoid therapy. Cytokine levels (pg/μg of free secretory component of immunoglobulin A) were compared between groups. We determined whether baseline cytokine levels modified treatment effect regarding subsequent need for systemic glucocorticoid therapy or occurrence of BPD (age 28 days).

Tracheal aspirates were obtained from 161 infants (77 receiving beclomethasone, 84 receiving placebo). Median IL-8 levels were lower in beclomethasone versus placebo infants on study days 8 (82.9 vs. 209.2, $P < 0.01$) and 15 (37.4 vs. 77.4, $P < 0.03$) after controlling for antenatal glucocorticoid therapy and maternal race. Median IL-1ra levels were lower in beclomethasone versus placebo infants only on study day 8 (86.5 vs. 153.3, $P < 0.01$). Fewer beclomethasone infants with baseline IL-8 levels in the interquartile range required systemic glucocorticoid therapy (beclomethasone 30.6% vs. placebo 65.8%, $P < 0.01$) or developed BPD (beclomethasone 42.4% vs. placebo 69.4%, $P < 0.03$).

We conclude that early-inhaled beclomethasone therapy was associated with a reduction in pulmonary inflammation after 1 week of therapy. Beclomethasone-treated infants with moderately elevated baseline IL-8 levels received less subsequent systemic glucocorticoid therapy and had a lower incidence of BPD than nontreated infants. **Pediatr Pulmonol. 2000; 30:275–281.** © 2000 Wiley-Liss, Inc.

Key words: interleukin-1 receptor antagonist; interleukin-8; inhaled glucocorticoid; oral glucocorticoid; preterm infants; pulmonary inflammation; bronchopulmonary dysplasia; outcome.

[1]Department of Pediatrics, Division of Newborn Medicine, The Floating Hospital for Children at New England Medical Center, Tufts University School of Medicine, Boston, Massachusetts.

[2]Division of Newborn Medicine, Pennsylvania Hospital, Philadelphia, Pennsylvania.

[3]Department of Epidemiology and Biostatistics, Boston University School of Public Health, Boston, Massachusetts.

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*Correspondence to: Cynthia H. Cole, M.D., M.P.H., Division of Newborn Medicine, Box 84, New England Medical Center, 750 Washington Street, Boston, MA 02111. E-mail: ccole@lifespan.org

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INTRODUCTION

Preterm infants are at higher risk for bronchopulmonary dysplasia (BPD) because pulmonary immaturity and exposure to factors such as volutrauma, barotrauma, free oxygen radicals, and infection.[1,2] Although a complete understanding of the exact mechanisms leading to the development of BPD remains unknown, pulmonary inflammation is a major contributing factor in its pathogenesis.[3,4] In addition to recruitment of inflammatory cells, one of the earliest events in inflammation is the local production of chemoactive agents such as inflammatory cytokines.[3,5,6] These cytokines increase the inflammatory response through positive feedback by further induction and amplification of peptide mediators. Inflammatory cytokines in tracheal aspirates such as intercellular adhesion molecule-1, tumor necrosis factor-α, interleukin-1β, interleukin-6, and interleukin-8 have been shown to be increased early in the course of preterm infants who subsequently develop BPD compared to those who do not.[3,4,5,7,8]

Systemic glucocorticoid therapy is widely used to prevent or treat BPD and has been shown to decrease pulmonary inflammation and reduce microvascular permeability.[9–11] Inhaled glucocorticoid therapy is increasingly used in the prevention and treatment of BPD because of its safety and efficacy as an antiinflammatory therapy for asthma in adults and children.[12–16] There are limited data on the safety and efficacy of inhaled glucocorticoid therapy in preterm ventilated infants. Two studies suggest that inhaled glucocorticoid therapy had no effect in reducing pulmonary inflammation in infants at risk for BPD.[14,17]

Our group has shown that early inhaled beclomethasone therapy in preterm infants at risk for BPD is associated with significantly less subsequent use of systemic glucocorticoid, less bronchodilator therapy, and less mechanical ventilation at 28 days of age, but that it does not prevent BPD.[18] In this study, we tested the hypothesis that early inhaled beclomethasone therapy reduces pulmonary inflammation in ventilated preterm infants at increased risk for BPD. We chose to measure interleukin-8 (IL-8) because this is one of the most potent and abundant neutrophil chemoattractant cytokines present in the lungs.[2] We also measured interleukin-1 receptor antagonist (IL-1ra), the release of which is mediated by a pro-inflammatory cytokine IL-1β and is the only naturally occurring antiinflammatory cytokine. Thus, elevation in IL-1ra levels reflects an anti-inflammatory response.[19] We also assessed whether study day 1 (baseline) IL-8 levels modified treatment effectiveness on other outcome variables such as subsequent use of systemic glucocorticoid therapy and development of BPD.

Abbreviations

BPD	Bronchopulmonary dysplasia
ELISA	Enzyme-linked immunosorbent assay
fSC	Free secretory component of immunoglobulin A
IL-1ra	Interleukin-1 receptor antagonist
IL-8	Interleukin-8
TA	Tracheal aspirate

MATERIALS AND METHODS

This study was part of a randomized, double-masked, placebo-controlled, multicenter trial, designed to study the safety and efficacy of inhaled beclomethasone therapy for the prevention of BPD. BPD was defined as supplemental oxygen requirement at 28 days of age and an abnormal chest radiograph as per the Edwards classification.[20] The institutional review board of each participating center approved the study. Informed consent was obtained from the parents.

The study subjects were ventilated preterm infants with birth weight ≤1,250 g, gestational age <33 weeks, and postnatal age 3 to 14 days. Infants were excluded if there was evidence of acute sepsis (clinical diagnosis and/or positive blood or cerebrospinal fluid culture), glucose intolerance (>120 mg/dL or >6.7 mmol/L), hypertension,[21] necrotizing enterocolitis,[22] abnormal renal function,[23] abnormal elevation of liver enzymes,[23] major congenital anomalies, or prior exposure to postnatal systemic glucocorticoid therapy.

Details of the randomization, calculation of the dose delivered, and procedures have been previously described.[18] The daily dose of beclomethasone dipropionate was tapered during 4 weeks from 1,000 to 125 μg/kg/day emitted from the metering valve. This is equivalent to a delivered dose of 40 to 5 μg/kg/day based on measurement of the dose exiting the endotracheal tube (4% of actuation dose). Actuations were equally divided among treatments per day. The study drug was delivered by metered dose inhaler attached to a 150-mL Aerochamber® (Monaghan Medical Corporation, Inc., Plattsburgh, NY), which was interposed between the infant's endotracheal tube and the anesthesia bag.

Sample Collection

Tracheal aspirate samples were obtained at two of the three centers participating in the study (Floating Hospital for Children at New England Medical Center, Boston, MA and Pennsylvania Hospital, Philadelphia, PA) from October 1993 to April 1997. Tracheal aspirates were obtained from infants who remained ventilated and had no exposure to systemic glucocorticoid therapy on study days 1 (baseline), 8, and 15 and on day 28 of life. Infants deemed to be clinically unstable for suctioning had no specimens collected on that day. Tracheal aspirates were

collected in Leuken's specimen traps (Sherwood Medical Co., St. Louis, MO) during patient care suctioning during the 12-hour period from 9 PM to 9 AM. After instillation of 1 mL of normal saline, a 5 F suction catheter was inserted slightly beyond the distal tip of the endotracheal tube, and all the secretions were aspirated. Three to five manual or ventilator breaths were given between instillation of saline and suctioning. The suction catheter was then flushed with 0.5 mL of normal saline. All specimens were immediately stored at 4°C and pooled after 12-h collection. The supernatant was separated from the pooled samples by centrifugation at 1,500 g for 10 min and small aliquots were stored at −70°C until assay for biochemical markers.

Cytokines

The concentrations of IL-8 and IL-1ra were measured with commercially available enzyme-linked immunosorbent assay (ELISA) kits (Quantikine kit, R & D Systems, Inc., Minneapolis, MN). A double-antibody sandwich immunoassay utilizing an immobilized monoclonal capture antibody and an enzyme-linked polyclonal antibody was used. The assays were performed in duplicate and a standard curve was constructed, using known amounts of recombinant IL-8 and IL-1ra. The plates were read at 450 nm on a micro-ELISA plate reader. The sensitivity was 3 pg/mL for IL-8 and 6.5 pg/mL for IL-1ra.

Free Secretory Component of IgA

The free secretory component of immunoglobulin A was used as a reference protein and was measured in the tracheal aspirate fluid, using the double sandwich direct ELISA of Chintalacharuvu et al.[24] with minor modifications. The standard was obtained from Dr. C. Kaetzel (Case Western Reserve University, Cleveland, OH). ELISA plates were coated with antibody, the samples were applied, and the bound secretory component was detected by alkaline phosphatase-linked antibody. The assays were performed in triplicate and a standard curve was constructed, using known amounts of free secretory component of immunoglobulin A (fSC). The plates were read at 405 nm on a micro-ELISA plate reader.

Outcome Measures

The primary outcome measures were tracheal aspirate levels of IL-8 and IL-1ra (pg/μg of fSC) collected on study days 1, 8, and 15, and on day 28 of life. We also assessed whether baseline markers of pulmonary inflammation (baseline IL-8 and IL-1ra) modified treatment effectiveness on other outcome measures such as subsequent use of systemic glucocorticoid therapy and development of BPD. To evaluate this relationship, we stratified our infants into three categories according to their baseline (study day 1) tracheal cytokine levels, as high (>75th percentile), medium (25th–75th percentiles), and low (<25th percentile).

Statistical Analysis

The effect of treatment on inflammatory markers was evaluated separately for study days 1, 8, 15, and day 28 of life. Because the tracheal aspirate samples were not collected from every patient at each of the four specified sampling times secondary to removal of the endotracheal tube, intolerance of suctioning, or initiation of systemic glucocorticoid therapy, a longitudinal analysis was not performed. We used the nonparametric Wilcoxon rank sum test for continuous variables because the data on inflammatory markers were not normally distributed. A linear regression model was used after transforming inflammatory marker data to logarithmic scale. We adjusted for antenatal glucocorticoid exposure and race in multiple linear regression models. Chi-square or Fisher exact tests were used for categorical variables. A result was considered significant if $P < 0.05$.

RESULTS

Patient Characteristics

From the two centers collecting tracheal aspirates, 207 of the 508 infants screened were eligible for the study. One hundred eighty-two infants were randomized and received study drugs. At least one or more tracheal aspirate samples were collected and analyzed in 161 infants (77 receiving beclomethasone, 84 receiving placebo). The primary reasons for failure to obtain any specimen from 21 infants (12 receiving beclomethasone, 9 receiving placebo) were either removal of the endotracheal tube within 24 hours after enrollment or a clinical assessment that the infant was too unstable to tolerate tracheal suctioning. Two samples were insufficient in volume for analysis, and one sample was lost in the storage and processing. The number of specimens processed on study days 1, 8, and 15 and on day 28 of life were 69, 45, 32, and 13 in the beclomethasone group and 78, 50, 37, and 19 in the placebo group, respectively. The groups were comparable in all baseline characteristics except for antenatal glucocorticoid use and race (Table 1).

Twelve infants in the beclomethasone group (15.6%) and 22 infants in the placebo group (26.2%) required subsequent systemic glucocorticoid therapy ($P = 0.03$). Thirty-five infants in the beclomethasone group (45.5%) and 41 infants in the placebo group (48.8%) subsequently developed BPD ($P = 0.30$).

Tracheal Aspirate IL-8 and IL-1ra Levels

IL-8 and IL-1ra levels (pg/μg of fSC, median and interquartile range) for study days 1, 8, and 15 are shown

TABLE 1.—Baseline Characteristics of the Infants in the Beclomethasone and Placebo Groups

Characteristic	Beclomethasone (N = 77)	Placebo (N = 84)
Birthweight (g)[1]	794 ± 179	793 ± 186
Gestational age (wk)[1]	25.8 ± 1.6	25.7 ± 1.8
Male gender (%)	49	54
Mother's race or ethnic group (%)*		
White	35	52
Black	33	33
Asian	9	4
Hispanic	10	7
Other	13	4
Age at enrollment (days)[1]	5.4 ± 3.5	5.1 ± 2.8
Any antenatal glucocorticoid exposure (%)*	88.3	76.2
Oxygenation index at entry[1]	3.7 ± 2.6	4.5 ± 4.2
Respiratory distress syndrome (%)	100	100
Surfactant therapy (%)	98.7	96.4

[1]Values are mean ± SD.
*$P < 0.05$.

in Figure 1. Baseline IL-8 levels (Fig. 1A) were comparable between study groups (beclomethasone 94.3, placebo 125 pg/μg of fSC, $P = 0.35$). One week after initiation of therapy, IL-8 levels were significantly lower in the beclomethasone-treated group compared to placebo (beclomethasone 82.9, placebo 209.2 pg/μg of fSC, $P < 0.01$) for crude analysis and also after adjusting for antenatal glucocorticoid use and maternal race. IL-8 levels on study day 15 were also significantly lower in beclomethasone-treated infants after controlling for race and antenatal glucocorticoid therapy on treatment effect (beclomethasone 37.4, placebo 77.4 pg/μg of fSC, $P < 0.03$) than in the placebo-treated babies. In the few infants who remained intubated on day 28 of life, there was no difference between study groups (beclomethasone 105.4, placebo 88.3 pg/μg of fSC, $P = 0.76$). IL-1ra levels also showed a pattern similar to IL-8 except for no significant difference between groups on study day 15 (Fig. 1B).

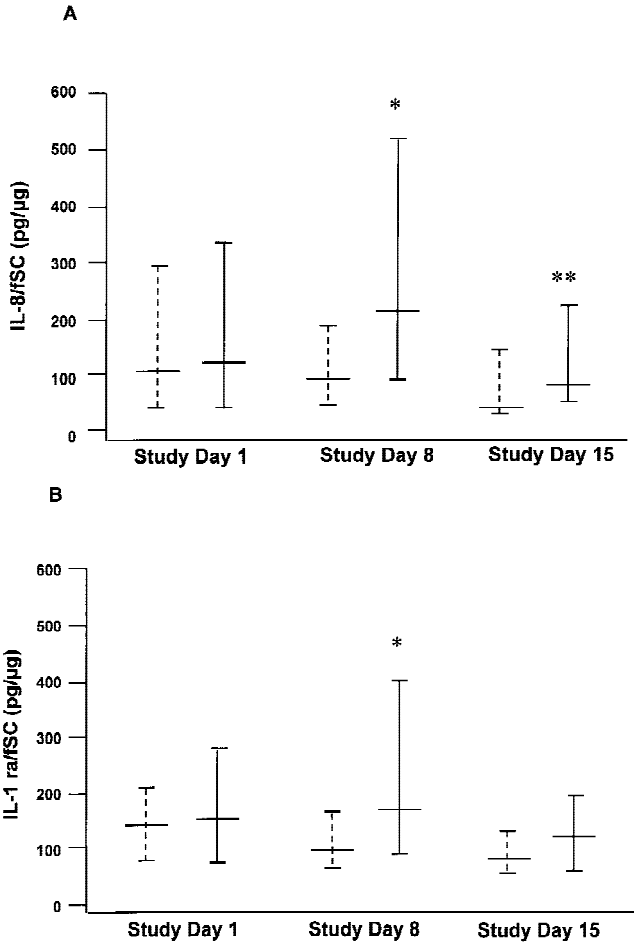

Fig. 1. IL-8 (A) and IL-1ra (B) cytokine levels in the tracheal aspirates obtained from infants in the two study groups. Median and 25th–75th percentile values in pg/μg of fSC are shown. $P < 0.01$ (*), adjusted $P < 0.03$ (**) between study groups, beclomethasone (dashed lines), and placebo (solid lines).

Influence of Baseline Tracheal Cytokine Levels on Treatment Effect

Several observations were made with respect to the relation between baseline pulmonary inflammation (based on study day 1 IL-8 and IL-1ra tracheal levels) and subsequent use of systemic glucocorticoid and development of BPD.

The proportion of infants requiring subsequent systemic glucocorticoid therapy increased in both groups when the baseline IL-8 levels were greater than the 25th percentile. Beclomethasone therapy was associated with less subsequent systemic glucocorticoid therapy than placebo treatment when the baseline IL-8 levels were in the interquartile range (beclomethasone 30.6% vs. placebo 65.8%, $P < 0.01$) (Fig. 2A).

With respect to the relation between baseline pulmonary inflammation and development of BPD, we found that the proportion of infants who developed BPD was less for beclomethasone-treated infants than placebo-treated infants whose baseline IL-8 levels were in the medium (interquartile) range (beclomethasone 42.4% vs. placebo 69.4%, $P < 0.03$) (Fig. 2B). Baseline IL-8 levels less than the 25th percentile or greater than the 75th percentile did not modify treatment effect in terms of subsequent use of systemic glucocorticoid therapy or development of BPD.

Baseline IL-1ra levels did not modify treatment effect with respect to either subsequent use of systemic glucocorticoid therapy or development of BPD. However, the trend in each quartile was toward less systemic glucocorticoid therapy in the beclomethasone compared to the placebo group (data not shown).

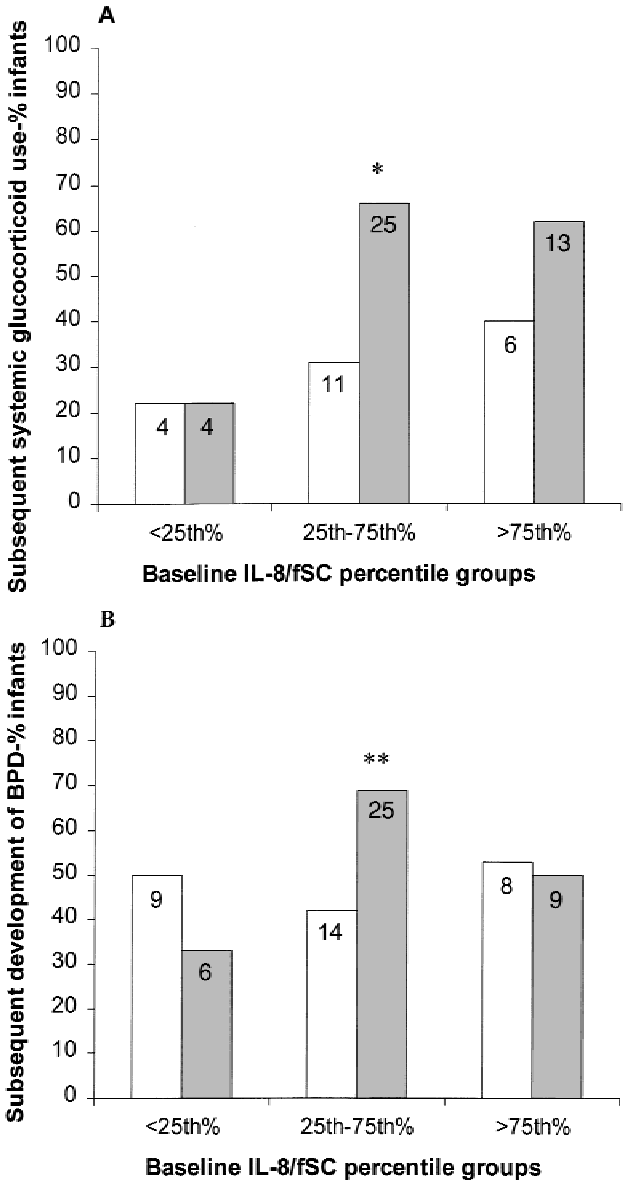

Fig. 2. Relation of study day 1 (baseline) IL-8/fSC percentile values in the two study groups with (A) subsequent use of systemic glucocorticoid and (B) subsequent development of bronchopulmonary dysplasia. Numbers in each bar represent number of infants in each group analyzed, $P < 0.01$ (*), $P < 0.03$ (**) between study groups, beclomethasone (open bars), and placebo (shaded bars).

DISCUSSION

Our data demonstrate that infants receiving inhaled beclomethasone therapy compared to placebo had significantly lower tracheal aspirate IL-8 and IL-1ra levels after 1 week of treatment and before tapering the beclomethasone dose. This is consistent with our hypothesis that early inhaled beclomethasone therapy reduces pulmonary inflammation. Although studies of systemic glucocorticoid therapy demonstrated reduction in tracheal markers of inflammation in infants at risk for BPD,[25–30] other investigators have not demonstrated a reduction of inflammation with inhaled glucocorticoid therapy. Groneck et al.[17] showed no significant reduction in inflammatory mediators in children receiving 2 weeks of inhaled beclomethasone therapy. Arnon et al.[14] showed no significant change in the bronchoalveolar lavage inflammatory cell counts in infants treated for 7 days with inhaled budesonide (1,200 μg/day) beginning at 14 days of age.

A pattern of an early increase and subsequent decrease in proinflammatory cytokine levels within the first month of life was reported by Groneck et al.[4] and Kotecha et al.[30] and was also observed in our placebo group. In each of our study groups, a slight increase in the inflammatory mediators in airway secretions was noted in the small number of infants who remained intubated on day 28 of life. This late increase may reflect persistent pulmonary inflammation in infants requiring prolonged mechanical ventilation. The observation that the antiinflammatory cytokine IL-1ra followed a trend similar to IL-8 suggests that with improvement in pulmonary inflammation, the antiinflammatory response to inflammation decreased.

Our data also demonstrate that the baseline IL-8 levels do modify the effect of treatment on outcome. Beclomethasone-treated infants compared to placebo-treated infants with baseline elevation of IL-8 greater than the 25th percentile had decreased needs for subsequent systemic glucocorticoid therapy, although the differences between groups were significant only when the IL-8 levels were in the moderate elevation (interquartile) range. Similarly, there was a significant difference between groups favoring beclomethasone therapy with respect to subsequent development of BPD when baseline IL-8 levels were moderately elevated (interquartile range), but not when the baseline IL-8 levels were less than the 25th percentile or greater than the 75th percentile. These combined observations of baseline pulmonary inflammation influencing the effectiveness of treatment (subsequent systemic glucocorticoid therapy) and the development of BPD suggest that infants with moderate baseline pulmonary inflammation may benefit from early inhaled beclomethasone therapy. Infants with little initial pulmonary inflammation (baseline IL-8 less than the 25th percentile) may not benefit from early inhaled glucocorticoid therapy. Infants with the highest degree of pulmonary inflammation (baseline IL-8 greater than the 75th percentile) may have a degree of inflammation too great to be controlled with this dosing regimen, formulation, or delivery of inhaled beclomethasone therapy.

One limitation of this study is that we could not sample tracheal aspirate specimens longitudinally from the entire original study population because infants who rapidly recovered had their endotracheal tube removed and sicker infants remained intubated. Therefore, infants

included in the study at any given study day were more likely to be at a greater risk for BPD compare to the entire study population. We also did not measure tracheal aspirate inflammatory mediators after initiation of systemic glucocorticoid therapy because inclusion of tracheal aspirates after exposure to systemic glucocorticoid would confound interpretation of results. Furthermore, the primary focus of this study was to assess the effect of inhaled beclomethasone on tracheal aspirate inflammatory mediators. It was not designed to compare tracheal aspirate inflammatory mediator response to inhaled versus systemic glucocorticoid therapy.

We did not exclude tracheal aspirate samples based on microbial colonization status. As previously reported,[18] we noted no difference in tracheal colonization or systemic infection between groups on any of the tracheal aspirate sampling days.

We conclude that early inhaled beclomethasone therapy can reduce pulmonary inflammation in infants at risk for BPD. This response may explain the decreased need for subsequent systemic glucocorticoid therapy and less development of BPD in infants who had moderate baseline elevations of IL-8. Further study of different dosing regimens, and improvement in aerosol delivery and in inhaled glucocorticoid formulations may result in even greater reduction in pulmonary inflammation and reduction in the severity of BPD.

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