Effect of Inhaled Corticosteroids on Markers of Pulmonary Inflammation and Lung Maturation in Preterm Infants With Evolving Chronic Lung Disease

Nehal A. Parikh, DO; Robert G. Locke, DO; Aaron Chidekel, MD; Kathleen H. Leef, RN, MSN; John Emberger, RRT; David A. Paul, MD; John L. Stefano, MD

Background: Chronic lung disease (CLD) is one of the most severely disabling conditions of extremely low-birthweight infants. Systemic corticosteroids are effective but cause many adverse effects. Targeted therapy with inhaled corticosteroids may be an effective and less toxic alternative.

Study Objective: To evaluate the additive effect of inhaled corticosteroids on markers of lung inflammation in infants receiving a 7-day course of systemic steroids.

Methods: Preterm neonates weighing 1 kg or less and aged 12 to 28 days who were prescribed a 7-day course of systemic corticosteroids for evolving CLD were studied prospectively and randomized to receive either a tapering 4-week course of beclomethasone metered-dose inhaler (MDI) (n = 5) or placebo MDI (n = 6). Primary outcome variables were the levels of pro- and anti-inflammatory cytokines, IL-8, TNF-α, IL-1α, and sIL-2R.

Results: This study was terminated early following literature reports of the adverse neurodevelopmental effects of dexamethasone. Measurements of respiratory and serum IL-8, IL-1α and TNF-α were similar between the study group taking inhaled and systemic corticosteroids and the study group taking systemic steroids alone. No differences were found between the two groups in relation to dynamic compliance or resistance.

Conclusions: The addition of inhaled corticosteroids to a 7-day systemic course of corticosteroids did not alter cytokine response or improve pulmonary function.

Chronic lung disease (CLD) is one of the most common diseases affecting extremely low-birth-weight (ELBW) infants, developing in approximately 30% of surviving infants who weigh less than 1 kg at birth. Lung injury occurs as a result of sustained lung inflammation, fibrosis, and altered remodeling that is a result of infection, mechanical injury, or high-inspired oxygen concentration. Extremely low-birth-weight infants are most at risk because of the high degree of immaturity of their lungs and inadequate adrenal response to illness. Improvements in neonatal care have greatly reduced the incidence of CLD in neonates older than 32 weeks gestation but have concurrently increased survival of ELBW neonates with CLD, resulting in no change or an increase in the incidence of the disease.

Systemic dexamethasone therapy, used to counteract the damaging effects of pulmonary inflammation, is associated with a number of adverse short-term multi-organ effects and long-term neurodevelopmental morbidities. Inhaled corticosteroids offer a targeted route of delivery that may minimize systemic adverse events. To date, most studies investigating the use of inhaled steroids in the preterm population have shown a good safety profile. Long-term data, though, are lacking, and a risk of hypothalamic-pituitary-adrenal-axis suppression exists. Short-term benefits include improved pulmonary compliance and airway conductance, fewer days on mechanical ventilation and supplemental oxygen, and reduced need for systemic steroids. Further studies with inhaled steroids are needed to investigate their mechanism of action in pulmonary inflammatory disease before large-scale trials are undertaken.

We performed a preliminary study to investigate whether the simultaneous initiation of inhaled corticosteroids added to a course of systemic corticosteroid therapy can attenuate levels of detrimental pulmonary cytokines in premature infants with early signs of CLD. The purpose of starting inhaled corticosteroids simultaneously is demonstrated by studies that have shown that inhaled steroids alone need a few days to display beneficial effect. We, therefore, hypothesized that inhaled corticosteroids provide persistent suppression of lung inflammatory cytokines by permitting sustained, direct anti-inflammatory therapy after the completion of a short course of systemic corticosteroids.
42 \mu g per actuation per methodology established by Cole and colleagues (n = 5).^{12} The placebo group received nonmedicated MDI in a similarly marked aerosol chamber using the same delivery technique (n = 6). The prescribed dose for beclomethasone for infants weighing 500 g to 750 g at randomization was 5 puffs every 8 hours for the first 14 days, followed by 5 puffs every 12 hours for the following 7 days, tapered to 5 puffs once a day for the last 7 days. Those infants weighing 751 g to 1 kg at randomization received 7 puffs every 8 hours for 14 days, followed by 7 puffs every 12 hours for the following 7 days, tapered to 7 puffs once a day for the last 7 days.

It was estimated that less than 4% per actuation dose of the study drug would be delivered to the trachea and lungs, as shown by previous studies.\textsuperscript{12,17,18} Based on this knowledge, the dosage regimen was estimated to deliver approximately 40 \mu g/kg daily at study entry, decreasing by 33% in two steps, down to a dosage of 13 \mu g/kg daily. The study design allowed for additional doses of systemic steroids to be administered at the discretion of attending physicians.

Baseline and once weekly tracheal aspirate (TA) fluid was obtained from intubated infants for infectious organisms along with sputum for measurement of interleukin-8 (IL-8), tumor necrosis factor-alpha (TNF-\alpha), interleukin-1 alpha (IL-1\alpha), soluble interleukin-2 receptor (sIL-2R), and secretory component of IgA (to normalize tracheal samples). In both intubated and nonintubated infants, serum for IL-8, TNF-\alpha, IL-1\alpha, and sIL-2R was obtained at the same time. Specimens were processed and frozen in a $-70^\circ$ freezer for batch-processing using established methodology at a later time. Noninvasive pulmonary function tests (Pulmonary Evaluation and Diagnosis System [PEDS], Medical Associated Services Inc, Hatfield, Pa) evaluating pulmonary compliance, resistance, and tidal volume measurements were obtained on all

### Methods

#### Eligibility

All ventilator-dependent ELBW infants (500 g to 1 kg) admitted to the Christiana Care Special Care Nursery in Newark, Del, from January 2000 to February 2001 were eligible for the study. Eligible infants were aged 12 days or older and were prescribed a course of systemic dexamethasone based on clinical need as determined by attending physicians, independent of this study protocol. Infants with major congenital anomalies of the cardiopulmonary or central nervous system, evidence of sepsis, prior postnatal glucocorticoid treatment, or necrotizing enterocolitis were excluded from the study. The Christiana Care Health System Institutional Review Board approved the study, and written parental informed consent was obtained.

#### Randomization

After informed consent was given by parents of eligible infants, those infants were randomly assigned by random number generation and sequentially numbered envelopes to receive either inhaled beclomethasone or inhaled placebo by a registered respiratory therapist (J.E.) who was not involved in the patients’ care. The clinical team, bedside nurses, and respiratory therapists were blinded to the treatment assignments.

#### Intervention

The systemic steroid regimen prescribed to study participants was a tapering 7-day course of 0.2 mg of dexamethasone per kilogram of body weight. All infants received the same 7-day course: 0.2 mg/kg for days 1 through 3, 0.1 mg/kg for days 4 through 6, and 0.05 mg/kg on day 7. In addition, infants randomized to the inhaled corticosteroid group concurrently received treatment with an inhaled beclomethasone dipropionate metered-dose inhaler (MDI) that provides

### Table 1

<table>
<thead>
<tr>
<th>Measure or Characteristic</th>
<th>Corticosteroid (Beclomethasone)</th>
<th>Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age, wk</td>
<td>24.8 ± 1.3</td>
<td>26.1 ± 1.1</td>
<td>.09</td>
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<tr>
<td>Birth weight, g</td>
<td>837 ± 168</td>
<td>847 ± 135</td>
<td>.91</td>
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<tr>
<td>Apgar score*</td>
<td>7 ± 1</td>
<td>7 ± 1</td>
<td>.68</td>
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<tr>
<td>SNAP† score</td>
<td>14.6 ± 6.8</td>
<td>16.5 ± 4.7</td>
<td>.60</td>
</tr>
</tbody>
</table>

* The Apgar score (Activity, Pulse, Grimace, Appearance, Respiration) reported here is the second one given by attending physicians after birth, at 5 minutes from birth. The Apgar score at 1 minute from birth is not provided.

† SNAP indicates Score for Neonatal Acute Physiology.

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intubated infants before starting the study drug and weekly thereafter for 4 weeks as per methodology previously described. All study infants were otherwise managed as per standard nursery care for preterm infants. A complete neurodevelopmental examination, including Bayley’s Scales of Infant Development, was scheduled for follow-up.

Outcomes

The primary outcome was a reduction in cytokine levels as related to pulmonary inflammation, including tracheal and serum IL-8, TNF-α, IL-1α, and sIL-2R as measured at the end of study drug period. Secondary outcomes included pulmonary mechanics, tracheal colonization patterns, additional need for systemic dexamethasone therapy, and duration of oxygen therapy.

Statistical Analysis

Sample size was estimated using a 2-tailed type-I error of 0.05, and β of 80%. A 30% difference in the adjusted inflammatory marker required a sample size of 22 patients. For most comparisons, a statistical significance was determined by a 2-tailed t test for continuous variables and by the Wilcoxon (Mann-Whitney U) rank sum test for categorical variables. Comparison of pulmonary function and cytokine levels over time was done by analysis of variance with repeated measures and by the Wilcoxon rank sum test for nonparametric analysis. Cytokine levels were adjusted for baseline values.

Results

Scientific evidence strongly suggestive of adverse neurodevelopmental effects of systemic dexamethasone appeared in the literature halfway into study enrollment, which prompted us to terminate the trial prematurely after enrollment of 5 infants randomized to beclomethasone and 6 infants to the placebo group. The subjects were well matched demographically for gestational age and birth weight, as well as Apgar (Activity, Pulse, Grimace, Appearance, Respiration) and SNAP (Score for Neonatal Acute Physiology) scores (Table 1). Baseline measures of mean airway pressure, dynamic compliance, resistance, and serum and respiratory cytokine levels were also similar at study entry (Table 2). Measurements of serum as well as respiratory IL-8, IL-1α, and TNF-α were similar between the inhaled corticosteroid and inhaled placebo groups. A representative graph of respiratory and serum TNF-α is presented in Figures 1 and 2. Respiratory sIL-2R was also not significantly different between the two groups.

Due to technical difficulties, we were unable to determine serum sIL-2R levels. Weekly measurements of dynamic compliance and resistance were similar between the two study groups. There was no difference in the need for subsequent doses of systemic steroids in the two groups. Time to extubation and duration of supplemental oxygen need were also similar between the two groups. There were no increased adverse events recorded with the additional use of inhaled corticosteroids. Infants in

Table 2

<table>
<thead>
<tr>
<th>Measure</th>
<th>Corticosteroid (Beclomethasone)</th>
<th>Placebo</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dynamic compliance</td>
<td>0.545 ± 0.187</td>
<td>0.535 ± 0.106</td>
<td>.91</td>
</tr>
<tr>
<td>Mean airway pressure</td>
<td>10.6 ± 1.5</td>
<td>11.5 ± 1.6</td>
<td>.39</td>
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<tr>
<td>Resistance</td>
<td>113 ± 38</td>
<td>124 ± 34</td>
<td>.63</td>
</tr>
<tr>
<td>Respiratory cytokine levels, pg/mL</td>
<td></td>
<td></td>
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<tr>
<td>Interleukin-1α</td>
<td>1.6 ± 1.9</td>
<td>0.8 ± 0.9</td>
<td>.40</td>
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<tr>
<td>Interleukin-2 receptor</td>
<td>10.8 ± 3.9</td>
<td>21.7 ± 18.5</td>
<td>.38</td>
</tr>
<tr>
<td>Interleukin-8</td>
<td>41.7 ± 27.2</td>
<td>97.3 ± 106.7</td>
<td>.29</td>
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<tr>
<td>Tumor necrosis factor-α</td>
<td>1.3 ± 1.5</td>
<td>0.6 ± 0.7</td>
<td>.38</td>
</tr>
<tr>
<td>Serum cytokine levels, pg/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interleukin-1α</td>
<td>46.0 ± 31.5</td>
<td>14.5 ± 18.4</td>
<td>.07</td>
</tr>
<tr>
<td>Interleukin-8</td>
<td>42.8 ± 31.2</td>
<td>77.8 ± 82.4</td>
<td>.39</td>
</tr>
<tr>
<td>Tumor necrosis factor-α</td>
<td>14.5 ± 21.8</td>
<td>5.3 ± 4.6</td>
<td>.33</td>
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</table>
both groups had some transient hyperglycemia that resolved after termination of the systemic corticosteroids. All infants are being followed in a developmental follow-up clinic.

Discussion

To our knowledge, this study is the first to evaluate the effects of simultaneous administration of inhaled and systemic corticosteroids by assessing markers of pulmonary inflammation in premature infants with early CLD. In addition, we concurrently measured serum cytokines, which allowed us to characterize systemic inflammation postextubation. Our results did not demonstrate an appreciable anti-inflammatory effect of inhaled corticosteroids when given in conjunction with systemic corticosteroids.

Recent prospective trials of inhaled steroids not simultaneously delivered with systemic steroids have also failed to demonstrate a sustained attenuation of proinflammatory pulmonary cytokines.\textsuperscript{15,23-25} Gupta et al\textsuperscript{25} measured IL-8 and IL-1ra in 161 infants who received inhaled glucocorticoids for 4 weeks beginning soon after birth. A reduction in the median levels of these cytokines at day 8 of the study was found, but no difference at days 15 or 28 was noted. The effect on proinflammatory cytokines was short-lived and possibly negated by the undesirable suppression of anti-inflammatory cytokines such as IL-1ra.\textsuperscript{25} Clinically, these authors as well as investigators for the OSECT (Open Study of Early Corticosteroid Treatment) trial in Europe, the largest trial of inhaled steroids in neonates, did not find any significant improvement in survival without CLD with the use of inhaled steroids.\textsuperscript{12,26}

We chose to evaluate alterations in IL-8, TNF-\(\alpha\), and sIL-2R because it has been shown that increased levels of these proinflammatory cytokines are detrimental to developing lungs.\textsuperscript{27} Several investigators have shown abnormally high levels of these cytokines in the tracheal fluid of infants who go on to have CLD.\textsuperscript{28-30} In the short-term, IL-1\(\alpha\) has been shown to improve acute endogenous surfactant production.\textsuperscript{31,32} Long-term consequences of IL-1\(\alpha\) overproduction, though, may not be beneficial.\textsuperscript{27} IL-1\(\alpha\) has been associated with oxidative stress injury, alveolar type II pneumocyte defects, and impaired blood oxygenation.\textsuperscript{33} It may also cause inappropriate accelerated lung maturation.\textsuperscript{33}

The major limitations of our study were an inadequate sample size and the high likelihood of a type II error. In light of the mounting evidence of adverse neurodevelopmental consequences with the use of systemic corticosteroids, we believed it would be unethical to continue the trial. Our nursery practice was changing in line with what was being recommended by several investigators and experts.\textsuperscript{10,22,34-36} In addition, several trials and two systematic reviews have not uncovered any beneficial effect of inhaled steroids on survival without CLD.\textsuperscript{12-15,23,24,37-41} Inconsistent and insufficient pulmonary drug delivery through the small airways of preterm infants when administration is carried out via MDI or nebulizer may in large part explain the lack of efficacy as compared to the systemic route.\textsuperscript{17} Efforts investigating improved methods of enhancing drug delivery have all yielded only
marginal benefits until recently. Newer delivery devices and smaller particle sizes offer promise in improving targeted steroid delivery.

There is a paucity of long-term pulmonary and neurodevelopmental outcome data following the use of inhaled steroids in ELBW neonates. Our small sample size precludes us from drawing any conclusions regarding long-term outcome. Early and prolonged use of inhaled steroids may place neonates at risk for pituitary adrenal suppression and pulmonary and neurodevelopmental adverse effects. The short-term beneficial effects of inhaled corticosteroids in the first 2 weeks of life are similar to those achieved by the systemic route at an older age. In those neonates treated via the systemic route after 2 weeks of life, acute benefits such as earlier time to extubation have not conferred any long-term benefits in pulmonary or neurodevelopmental morbidities. An increasing number of trials are alerting us to the adverse effects of systemic dexamethasone on the immature central nervous system. A 2001 metanalysis by Barrington on the neurodevelopmental effects of postnatal steroids in preterm infants underscores the increased prevalence of neurodevelopmental disability (relative risk, 1.66; 95% CI, 1.26, 2.19) and cerebral palsy (relative risk, 2.86; 95% CI, 1.95, 4.19) in systemically treated infants upon long-term follow-up. The current practice is to limit the use of these powerful drugs to exceptional clinical circumstances with full parental informed consent before initiation of therapy.

Research investigating the role of high-frequency ventilation (HFV) to prevent CLD by minimizing the volutrauma associated with conventional modes of ventilation has also been fraught with controversy. Earlier trials were plagued by difficulties of inadequate lung recruitment, frequent crossover, and relative inexperience in some centers with HFV. Similar rates of CLD in both studies and a significant increased incidence of severe intracranial hemorrhage in two of the trials were the end result. More recent trials in the post–surfactant era with improved lung recruitment strategies have culminated in four studies that demonstrated no difference and one additional study that used high ventilator pressures with conventional ventilation showing improved rates of CLD in an HFV group. The two largest, and arguably the best designed, trials of early HFV in preterm infants to prevent CLD were recently completed. Unfortunately, their conflicting results still leave doubt about the role of HFV in preventing CLD. Using a rigorously defined protocol, Courtney et al demonstrated an absolute difference of 9% in improving survival without CLD (96% vs 47% in control group). Johnson et al found no difference in their population of preterm infants born at less than 28 weeks gestation. The trial by Courtney et al suggest that in the hands of experienced clinicians and a well-defined protocol, HFV offers some advantages. In most circumstances, however, the evidence indicates that the mode of ventilation does not impact CLD-free survival.

Figure 2. Weekly measures of serum cytokine TNF-α levels (pg/mL) in the two study groups.
Another promising therapy on the horizon for high-risk ELBW infants is inhaled nitric oxide (iNO). In preterm infants, iNO improves oxygenation by decreasing pulmonary vascular resistance and possibly by attenuating neutrophil accumulation and sequestration in the lung. One 1999 trial, using a low dose of 5 ppm, demonstrated a benefit in 80 preterm infants with severe hypoxic respiratory failure. The infants receiving iNO spent fewer days on ventilators and had a lower incidence of CLD, with no increased risk or progression of intracranial hemorrhage.57 No benefit in survival or CLD was noted in two other trials, however.58,59 In addition, long-term pulmonary and neurodevelopmental outcomes are as yet unknown. Therefore, iNO therapy cannot be recommended until multicenter, randomized trials—which are already under way—can demonstrate unequivocally the benefit of iNO on long-term pulmonary outcomes without an increase in neurodevelopmental morbidities.

Chronic lung disease is a multifactorial disease that is not amenable to any single intervention. Inhaled steroids may be useful for the reactive airway disease component of established CLD. However, the clinical use of high-dose, prolonged-course inhaled steroids to ameliorate developing lung disease in ELBW neonates does not appear warranted at this time.

References


