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COMMENTARY

CC10 Administration to Premature Infants: In Search of the "Silver Bullet" to Prevent Lung Inflammation

Commentary on the article by Levine *et al.* on page 15

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In this issue of *Pediatric Research,* Levine and co-workers (1) report their results in the article entitled, "The Safety, Pharmacokinetics, and Anti-inflammatory Effects of Intratracheal Recombinant Human Clara Cell Protein in Premature Infants with Respiratory Distress Syndrome." The authors administered a single dose of Clara cell protein (CC10) to premature infants shortly after birth and they found that the administration was associated with lower numbers of leukocytes and neutrophils isolated from tracheal aspirate samples than from infants receiving placebo. In addition, tracheal aspirate protein concentrations were lower in infants receiving CC10 than in those receiving placebo. This pilot study addresses the effect of CC10 on lung inflammatory markers because there is abundant evidence that lung inflammation participates in the pathophysiology of bronchopulmonary dysplasia (BPD) in premature infants.

NEW BPD: PREVALENCE AND PATHOPHYSIOLOGY

BPD is still a common complication of prematurity despite improvements in the acute management of premature infants. The National Institute of Child Health and Human Development (NICHD) Neonatal Research Network reported the incidence of BPD to be 52% in infants born between 500 and 750 g and 34% in infants born between 750 and 1000 g (2). Aside from poor short- and long-term pulmonary outcomes associated with the development of BPD (3,4), the development of BPD is independently associated with a higher risk of poor developmental outcomes in premature infants than in premature infants who do not develop BPD (5). The poor developmental outcome is likely a result of a generalized inflammatory state in premature infants occurring in the perinatal or early postnatal stage that leads to the development of BPD and may also increase the susceptibility for CNS damage. Thus, developing rational strategies to prevent the development of BPD or, at the very least, the severity of the disorder is an appropriate priority for clinical research and the focus may be even more appropriately directed against the generalized inflammatory state than the lung.

To design rational interventions against the development of BPD, defining the clinical and pathologic end points as well as relevant pathophysiology is crucial. The most common clinical end point is the requirement for supplemental oxygen at 36 wk postconceptual age, a reasonable end point for assessing pulmonary function. Levine and co-workers used this clinical end point but did not have the power, and perhaps the study design to adequately assess the intervention against this end point. Thus, end points proximal to the development of BPD, which might indicate relevant pathophysiology, were chosen by the authors. The pathogenesis for the development of BPD has not been firmly established, but lung injury caused by inflammatory cell infiltration of the lung and oxidative injury to the lung both independent of and dependent on lung inflammation are still the most prevalent factors thought to be proximal events in the development of BPD (6). The inflammatory cell infiltration into the lung and lung oxidative stress are imposed on the extremely immature lung that is poorly prepared for these injurious elements.

BPD AND LUNG INFLAMMATION

The literature suggesting that lung inflammation contributes to the development of BPD is relatively strong. First, maternal chorioamnionitis before the delivery of a premature infant was associated with an increased risk for the development of BPD in premature infants (7), and the clinical findings in premature infants consistently indicate that the processes are frequently underway in the first day or days of life (8,9). In addition, trials of postnatal steroids found a reduction in the incidence of BPD but with an unfortunate association with a poorer developmental outcome than in the control group (10). These findings suggest that interventions targeting inflammation may reduce the incidence of BPD but that steroids are not specific enough to limit deleterious effects in other organs.

INTERVENTIONS IN BPD: THE SEARCH FOR THE SILVER BULLET

CC10, which was studied by Levine *et al.* and reported on here, represents an excellent possible "silver bullet," address-

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ing lung inflammation in premature infants. CC10 is a small molecule made by Clara cells in the lung and has potent effects *in vitro,* including the inhibition of phospholipase A2 (PLA2) and fibronectin binding (11–13). These effects, if they were to occur *in vivo,* would probably inhibit inflammatory and fibrotic responses, respectively. CC10 deficiency has been associated with high expressions of cytokines in the lung and infiltration of the lung with inflammatory cells in animal models of lung inflammation (14,15), and the delivery of recombinant CC10 has provided protection in animal models of lung injury (16). These findings provide a strong rationale for considering CC10 administration in humans deficient in the expression of CC10.

The decision to design studies in which CC10 is administered to premature infants is strongly supported by studies indicating that these infants may be developmentally deficient in the expression of this protein. Lassus *et al.* (17) found that CC10 expression increases as a function of gestational and postconceptual age. Ramsay *et al.* (18) extended the studies of CC10 in premature infants, determining that infants who developed BPD had lower levels of CC10 in their tracheal aspirates than premature infants who did not develop BPD. The differences in CC10 levels in the tracheal aspirates between the two groups were noted on the first day of life and became even more marked at the end of the first week.

The present study of CC10 administration has a strong rationale and is well designed to address the hypothesis that CC10 administration was safe and was associated with evidence of less lung inflammation than administration of placebo. Ramsay and co-workers observed that premature infants susceptible to the development of BPD have lower levels of tracheal aspirate CC10 on the first day of life than infants who do not develop BPD, so that administration of CC10 on the first day of life was wise. The CC10 expressions in the tracheal aspirates over the first week in the present study are interesting. In the placebo-treated group, the CC10 levels increased over the first week of life, which is a pattern that was observed in infants, reported by Ramsay *et al.,* who did not develop BPD. A little more troubling is the pattern in infants treated with CC10. In both the CC10-treated groups, the levels of CC10 were high on d 1 of life after administration and at the very best were not induced over the first week, and in the higher-dose group the levels were actually lower at the end of the first week than the group given placebo. This interesting finding suggests that CC10 administration may down-regulate the expression of the endogenous gene, which has not been reported and is worth some investigation by itself. Alternatively, the lack of induction of CC10 in patients given the recombinant protein may also indicate a change in the phenotype of Clara cells in the lung or perhaps some unexpected damage to Clara cells with the consequent decrease in CC10 levels in the tracheal aspirates. These possibilities should be studied further. The findings of decreased CC10 levels after administrations and the pharmacokinetic data reported here by Levine *et al.* suggest that premature infants may need repeated doses to maintain relatively high CC10 levels in bronchoalveolar fluid. However, there is a clinical initiative to extubate premature infants as quickly as possible to nasal continuous positive airway pressure (CPAP), and, thus, repeated intratracheal dosing may not be realistic in all patients. Some of these infants will develop BPD despite being extubated. Systemic administrations of CC10 do get the protein distributed to the bronchoalveolar space and may be a reasonable alternative to intratracheal administrations in extubated premature infants (A. Pilon, personal communication). Repeated administrations should also be investigated further.

The study by Levine *et al.* was not powered to detect a difference in the incidence of BPD nor evidence of possible toxicities. Although concerns about toxicity are relevant, the fact that CC10 is a molecule expressed endogenously and is eliminated rapidly lessens the concerns about toxicities, making this intervention a seemingly safe candidate addressing inflammation and BPD. Because a decrease in BPD was not a realistic end point in this study, the authors measured markers of inflammation and injury, which were tracheal aspirate neutrophil counts and total protein concentrations, respectively. Tracheal aspirate neutrophil counts have been observed to be elevated early in the course of infants that developed BPD (19), so that the lower counts in infants given CC10 are a promising indication that CC10 limited lung inflammatory cell infiltration. Furthermore, the decrease in the tracheal aspirate protein concentration is a reasonable biomarker indicating a decrease in the lung injury in premature infants given CC10, and the finding is promising. This same laboratory observed a similar decrease in tracheal aspirate albumin concentrations in premature infants dosed with recombinant human superoxide dismutase in a pilot study (20). A larger study of this recombinant molecule did not find a decrease in BPD in premature infants but did see a decrease in wheezing episodes in infants dosed with superoxide dismutase (21). It is reasonable to interpret the decrease in tracheal aspirate neutrophil counts and protein concentrations as an indication that CC10 administration diminished the acute lung injury sequence that is important in the development of long-term pulmonary morbidity, but that the development of BPD may be influenced by many other variables, including clinical care practices (22), so that using BPD as an end point for CC10 effects may not reflect the true impact of the intervention on lung development and function.

HOW MIGHT CC10 WORK IN PREMATURE INFANTS?

The specific mechanisms of effects of CC10 *in vivo* have not been clarified, so how CC10 could work in premature infants is not clear. Perhaps the best study *in vivo* addressing deficiency of CC10 on lung inflammation and CC10 replacement *in vivo* was recently published by Wang *et al.* (16). They found that mice deficient in CC10 had higher lavage leukocyte and neutrophil counts than wild-type mice when infected with respiratory syncytial virus. The CC10-deficient mice also had evidence of an exaggerated sensitization-induced bronchoconstriction and they cleared RSV less well than their wild-type counterparts. Particularly noteworthy is the fact that administration of recombinant CC10 to the deficient mice reversed the effects of the deficiency. These findings are noteworthy in that they indicated findings in end points (bronchoalveolar lavage neutrophil counts) that were similar to those found in the present study, and CC10 adminis-

tration improved the elimination of the pathogen and reversed the effects on lung inflammation assessed histologically.

CC10 inhibits PLA2 function and fibronectin binding *in vitro*. Is there any evidence that these pathways may be relevant in premature infants who develop BPD? Fibronectin has been studied in premature infants with respiratory distress syndrome, and higher levels have been noted in the tracheal fluids in infants who developed BPD (23). Although this does not indicate a cause-and-effect relationship, it is suggestive that fibronectin may play a role in altered lung development and/or fibrosis in premature infants who develop BPD. It is intriguing to speculate that CC10 administration could lead to fibronectin binding with inhibition of fibronectin-mediated lung processes contributing to BPD. There have been no reports, however, that fibronectin expression or deposition in tissues contributes to acute inflammatory process, and, therefore, the early decrease in tracheal aspirate neutrophil counts are probably not due to binding of CC10 to fibronectin. PLA2 activities have not been studied in premature infants. However, PLA2 has been shown to lead to surfactant inactivation (24) and to be pro-inflammatory in models of lung inflammation (12,13). Interestingly, in an animal model of volutrauma-induced lung injury, the injury was greater in CC10-deficient mice than in wild-type mice, and PLA2 inhibition decreased lung injury in both the deficient and wild-type mice. Furthermore, in the same study, CC10-deficient mice had higher PLA2 activities than did wild-type mice. Thus, inhibition of PLA2 is a reasonable speculation for CC10 function *in vivo*. It would be helpful if these and other pathways were studied in premature infants dosed with CC10.

In summary, the study reported by Levine and co-workers in this issue of *Pediatric Research* is well designed, and the fact that CC10 administration was associated with lower tracheal aspirate neutrophil counts and lower tracheal aspirate protein concentrations is promising. The rapid elimination of CC10 and the association with lower tracheal aspirate levels of CC10 after elimination of the administered protein suggests that more than one dose may be necessary. The findings in this study are exciting because they indicate that optimal CC10 dosing may be an extremely specific intervention against lung inflammation and may thereby maximize the risk/benefit ratio of this antiinflammatory intervention. The study reported herein should lead to additional trials, which will probably need to include multiple centers to determine optimal dosing regimens and the effect of the treatment on the development of BPD and other long-term pulmonary end points.

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