

A Pilot Study to Determine the Safety and Feasibility of Oropharyngeal Administration of Own Mother's Colostrum to Extremely Low-Birth-Weight Infants

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ABSTRACT

Own mother's colostrum is rich in cytokines and other immune agents that may stimulate oropharyngeal-associated lymphoid tissue if administered oropharyngeally to extremely low-birth-weight (ELBW) infants during the first days of life when enteral feeding is contraindicated. However, the safety and feasibility of the oropharyngeal route for the administration of colostrum have not been determined.

PURPOSE: To determine the safety of oropharyngeal administration of own mother's colostrum to ELBW infants in first days of life. A secondary purpose was to investigate the feasibility of (1) delivering this intervention to ELBW infants in the first days of life and (2) measuring concentrations of secretory immunoglobulin A and lactoferrin in tracheal aspirate secretions and urine of these infants.

SUBJECTS: Five ELBW infants (mean birth weight and gestational age = 657 g and 25.5 weeks, respectively).

DESIGN: Quasi-experimental, 1 group, pretest-posttest design.

METHODS: Subjects received 0.2 mL of own mother's colostrum administered oropharyngeally every 2 hours for 48 consecutive hours, beginning at 48 hours of life. Concentrations of secretory immunoglobulin A and lactoferrin were measured in tracheal aspirates and urine of each subject at baseline, at the completion of the intervention and again 2 weeks later.

RESULTS: All infants completed the entire treatment protocol, each receiving 24 treatments. A total of 15 urine specimens were collected and 14 were sufficient in volume for analysis. A total of 15 tracheal aspirates were collected, but only 7 specimens (47%) were sufficient in volume for analysis. There was wide variation in concentrations of secretory immunoglobulin A and lactoferrin in urine and tracheal aspirates among the 5 infants; however, several results were outside the limits of assay detection. All infants began to suck on the endotracheal tube during the administration of colostrum drops. Oxygen saturation measures remained stable or increased slightly during each of the treatment sessions. There were no episodes of apnea, bradycardia, hypotension, or other adverse effects associated with the administration of colostrum.

CONCLUSIONS: Oropharyngeal administration of own mother's colostrum is easy, inexpensive, and well-tolerated by even the smallest and sickest ELBW infants. Future research should continue to examine the optimal procedure for measuring the direct immune effects of this therapy, as well as the clinical outcomes such as infections, particularly ventilator-associated pneumonia.

KEY WORDS: extremely low birth weight, human milk, immunotherapy, oropharyngeal administration of colostrum

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Dramatic advances in neonatal medicine over recent decades have resulted in decreased mortality for extremely low-birth-weight (ELBW; birth weight [BW] <1000 g) infants. Approximately 80% of infants born at 24 weeks of gestation will survive to discharge, compared with 50% just 15 years ago.¹ However, the survival of these infants is associated with significant short- and long-term morbidity, including nosocomial infection,^{2,3} necrotizing enterocolitis,⁴ intraventricular hemorrhage,^{4,5} periventricular leukomalacia,⁶ and adverse long-term neurodevelopmental sequelae including cognitive and motor disabilities, visual and/or hearing impairment, and cerebral palsy.⁷⁻⁹

Nosocomial infection, an acquired infection after 48 hours of hospital admission, is particularly important because of its high prevalence and its association with other morbidities including poor growth,^{10,11} adverse long-term neurological sequelae,¹⁰ increased length of hospital stay,¹² and a substantial cost¹² to families, hospitals, and society. The risk of acquiring a nosocomial infection is inversely related to birth weight and gestational age and directly related to the severity of illness at birth.² The ELBW infant is therefore at highest risk, with recent data showing that up to 65% of ELBW infants will have at least 1 infection during their neonatal intensive care unit (NICU) stay.¹⁰

Nosocomial infection of the bloodstream (bacteremia) is especially common in ELBW infants, primarily as a result of long-term vascular access for parenteral nutrition. A single bloodstream infection extends the length of hospital stay by 7 days for ELBW infants and costs an additional \$5875 for infants weighing 401 to 750 g and \$12 480 for infant weighing 751 to 1000 g.¹² Using recent birth data¹³ and assuming a 30% rate of bacteremia^{10,14} per ELBW infant born in the United States, the estimated annual cost is \$56 400 000 to \$119 808 000 per year. However, many ELBW infants have *several* cases of bacteremia during their 3- to 4-month hospitalization, thereby increasing these baseline cost estimates.

The risks of acquiring a nosocomial infection are modifiable through the use of own mother's milk (OMM) feedings for the ELBW infant in the NICU. Research has linked OMM feedings with a lower incidence of nosocomial infections¹⁵⁻¹⁹ in very low birth-weight (BW < 1500 g) infants compared with formula-fed infants. Although these studies have not focused specifically on the ELBW population, evidence from biochemical and immunological studies suggest that OMM feedings, especially colostrum, may provide the highest level of protection from nosocomial infection for the least mature ELBW infants.²⁰⁻²⁴ Unfortunately, clinical instability typically precludes enteral feedings in the first days of life for ELBW infants and alternative methods for administering colostrum have not been investigated.

The purpose of this study was to determine the safety of oropharyngeal administration of own mother's colostrum to ELBW infants in first days of life. A secondary purpose was to investigate the feasibility of (1) delivering this intervention to ELBW infants in the first days of life and (2) measuring concentrations of secretory immunoglobulin A (sIgA) and lactoferrin in tracheal aspirate secretions and urine of these infants.

BACKGROUND

Immune Protection in Own Mother's Colostrum

The comprehensive immune protection derived from OMM is attributed to a multitude of immunologically derived factors that provide antimicrobial and anti-inflammatory protection against infection and modulate the recipient infant's immune system.²⁵ Recent studies suggest an inverse relationship between the concentration of protective immune factors in colostrum and the duration of pregnancy.²⁰⁻²⁴ Thus, many of these protective factors are more highly concentrated in the colostrum of women who deliver ELBW infants, which suggests that these factors have an important biological role in protecting the recipient infant during the first days of life and that the first days of life may be a critical exposure period for the ELBW infant.

On the basis of these findings, preterm colostrum may be especially protective during the first days of life when ELBW infants are the sickest and at highest risk for acquiring an infection. However, the underdeveloped gastrointestinal tract and the presence of prematurity-associated morbidities that compromise gastrointestinal perfusion typically preclude enteral feedings during this period. The prolonged inability to feed enterally can lead to intestinal atrophy,²⁶ which increases the risk of localized inflammation, feeding intolerance, necrotizing enterocolitis, and nosocomial infections. Thus, there is a critical need to identify safe and efficacious alternatives for administering preterm colostrum as a potential "immune therapy" to ELBW infants in the first days of life when they cannot be fed enterally.

Oropharyngeal Administration of Colostrum

An alternative method of administering colostrum is by the oropharyngeal route. This involves placing small amounts of the colostrum directly onto the oral mucosa with the expectation that the colostrum or selected components are absorbed by the mucous membranes.²⁷ In addition, cytokines present in colostrum (colostral cytokines) may interact with lymphoid cells within the recipient infant's oropharyngeal associated mucosal tissues,^{28,29} while human milk oligosaccharides (HMOs) may provide local

barrier protection, on the basis of their ability to inhibit the adhesion of pathogens to epithelial cell surface receptors.^{30,31} The oropharyngeal route has been used to administer immunomodulating agents under other circumstances. For example, interferon- α , an immune cell-derived cytokine, has been administered safely and effectively to adult human subjects,³²⁻³⁶ using the oropharyngeal route.

A recent review²⁷ supports the concept that own mother's colostrum, when administered oropharyngeally, may serve as a potential immune therapy for the ELBW infant. On the basis of this evidence, it is plausible that oropharyngeally administered colostrum may evoke systemic immunostimulatory effects and potentially protect against nosocomial infection, including pneumonia.

Most of the research pertaining to colostrum cytokines has focused on their role within the gastrointestinal tract. However, during feeding at the breast, the exogenous cytokines in mother's milk may stimulate the infant's oropharyngeal-associated lymphoid tissue and gut-associated lymphoid tissue, in a synergistic manner, with a combined response potentially greater than that for a single site of stimulation. Separate work has suggested that the HMOs interfere with adhesion of bacteria in the oral cavity,³⁷ which translates into reduction in risk for otitis media and lower respiratory tract infections. The term *breastfed infant* would be the recipient of this combined effect. However, for the ELBW infant in the NICU who receives enteral feedings, such as minimal volume feedings, via a nasogastric tube, colostrum cytokines and HMOs would not have contact with oropharyngeal-associated lymphoid tissue or mucous membranes in the oral cavity. This deficit could be corrected with the oropharyngeal administration of colostrum, and as such, it would be considered a complement rather than a substitute for minimal volume (trophic) feedings.

Theoretically, oropharyngeal administration of colostrum would provide local barrier protection and change levels of immunologically derived factors, such as sIgA and lactoferrin, in bodily fluids. The potential influence of these factors on immune system integrity in ELBW infants is described next.

Immunologically Derived Factors

Secretory immunoglobulin A is an antibody that inhibits attachment of pathogens to the respiratory and intestinal mucosal epithelial barrier,³⁸ maintains mucosal intestinal integrity, and provides specific barrier protection against pathogens that cause respiratory and gastrointestinal tract infections.^{23,38-40} Interleukin-6, a cytokine in mother's milk, stimulates the growth and differentiation of B lymphocytes into IgA-secreting plasma cells.⁴¹ Theoretically, oropharyngeal administration of colostrum interleukin-6 may stimulate sIgA production, possibly resulting in

higher concentrations of sIgA in tracheal secretions and urine.

Lactoferrin is a glycosylated, iron-binding protein with potent bacteriocidal, bacteriostatic, antiviral, anti-inflammatory, and immunomodulatory properties.^{24,42-45} Like sIgA, lactoferrin is highly concentrated in colostrum,^{44,46} resistant to digestive enzymes,⁴³ and excreted at higher levels in urine and stool of mother's milk-fed infants, as compared with those of formula-fed cohorts.^{43,47,48} The appearance of lactoferrin in the urine suggests that it is systemically absorbed and may influence the infant's systemic response to infection.^{49,50} Lactoferrin may also protect against infection by inhibiting the attachment of pathogenic bacteria to cells lining the oropharyngeal mucosa, and, if swallowed, may provide similar barrier protection in the gastrointestinal tract.⁵¹

In combination, sIgA, lactoferrin, and HMOs provide barrier protection against respiratory pathogens that may penetrate the mucosa of the upper respiratory tract and cause ventilator-associated pneumonia (VAP) in ELBW infants. As the second most common nosocomial infection in the United States, VAP is associated with a significantly prolonged length of stay, considerable costs, and high mortality rate for extremely premature infants.⁵² Prevention of VAP in ELBW infants is therefore a clinical priority; however, the majority of commercially available oral care products contain chemicals that may be unsafe for ELBW infants. Even those that contain antibacterial components naturally occurring in human milk have not been well tested for safety and efficacy in the ELBW population. However, the use of own mother's colostrum via the nasogastric route for ELBW infants is safe, inexpensive, readily available, and not associated with any adverse effects to the recipient infant. This study was designed to determine the safety and feasibility of administering colostrum via the oropharyngeal route.

METHODS

Design

This pilot study used a quasi-experimental, 1 group, pretest-posttest design. The independent variable was the oropharyngeal administration of own mother's colostrum. The dependent variables were sIgA and lactoferrin concentrations in the infant's tracheal aspirates and urine before and after treatment with colostrum.

Subjects and Setting

Data were collected from infants cared for in a 44-bed tertiary NICU, who met the following inclusion criteria: BW < 1000 g, less than 28 weeks of gestation, and appropriate for gestational age. Exclusion criteria were infants with congenital anomalies, gastrointestinal disorders, renal disorders, receipt of vasopressor medications more than 10 $\mu\text{g kg}^{-1} \text{ minute}^{-1}$, maternal

history of substance abuse, or positive maternal HIV status. The study was approved by the institutional review board of the institutions where data were collected and where the principal investigator was a doctoral student.

This pilot study employed a labor-intensive protocol and necessitated the antenatal recruitment of ELBW infants who would serve as subjects. Maternal informed consent was obtained prior to the infant's birth. Seventeen mothers were approached and 15 agreed to participate. However, of these 15 women, 10 (67%) sustained their pregnancies beyond 28 weeks and were no longer eligible to participate. The remaining 5 mothers served as subjects for this study.

Five ELBW infants participated in this study. The mean birth weight was 657 g (range, 585-725 g). The mean gestational age was 25.5 weeks (range, 24³/₇-28³/₇ weeks). Two infants were white, 2 were African American, and 1 was Middle Eastern. There were 3 males and 2 females.

Measures

Secretory immunoglobulin A and lactoferrin were measured in the infant's tracheal aspirates and urine, before and after the oropharyngeal administration of colostrum.

A higher concentration of these immune products in tracheal aspirate secretions would suggest enhanced local mucosal immune protection, whereas elevated concentrations in urine would be consistent with enhanced systemic protection against infection.

Commercially available enzyme-linked immunosorbent assay kits were used to measure sIgA (ALPCO Diagnostics, Windham, New Hampshire) and lactoferrin (Calbiochem-EMD Biosciences Inc, San Diego, California) concentrations. Results were determined using a standard curve as the point of reference. Prior to analysis, the specimens were thawed and volumes measured, and they were then centrifuged at 1500 rpm for 30 minutes.

Procedures

Maternal informed consent for study participation was secured prior to the infant's birth. A baseline tracheal aspirate specimen was obtained in the delivery room prior to the administration of surfactant.

Collection of Colostrum

Within 24 hours after delivery, the principal investigator met with each mother enrolled in the study and explained the standardized protocol for milk removal utilizing a hospital-grade electric breast pump (Symphony, Medela, Inc, McHenry, Illinois) and demonstrated hand expression of colostrum. Mothers were encouraged to pump using a double pump collection kit every 2 to 3 hours for a total of 8 times per 24-hour period. Mothers received these instructions both verbally and in written form.

During this meeting, the principal investigator gave each mother a supply of 6 sterile, pre-labeled specimen cups. Mothers were instructed to manually express 1.0 mL of colostrum into each cup as soon as possible after delivery. Mothers were reminded to wash their hands before expressing milk, and especially before handling the small cups that would be utilized for colostrum storage. They were instructed to send all colostrum specimens to the NICU for immediate refrigeration.

Preparation of Syringes

The syringes that would be used to oropharyngeally administer the colostrum were prepared by the principal investigator when at least 2.4 mL of own mother's colostrum was available in the NICU. Although a total of 4.8 mL of colostrum was necessary to provide colostrum drops to the infant for the entire 48-hour treatment period, 2.4 mL was sufficient to initiate the protocol and provide drops for the first 24 hours of the treatment period. Using sterile gloves and sterile technique, the principal investigator filled each of 12 sterile, needle-less tuberculin syringes with 0.2 mL of own mother's colostrum. The syringes were labeled with the infant's preprinted hospital label that included the medical record number and patient name, and with the date and time of sample preparation. The labeled syringes were placed in a pre-labeled plastic container and refrigerated in the infant's room. An additional 12 syringes, for the second 24 hours of the treatment protocol, were prepared by the principal investigator in the same manner when another 2.4 mL of own mother's colostrum was available.

Delivery of Intervention During Treatment Protocol

Once the first 12 syringes were prepared, the infant's nurse obtained a baseline urine specimen. Then 2 nurses checked the infant's name against the label on the pre-filled syringe. The syringe was placed in warm water for 5 minutes to bring the colostrum to room temperature. The infant's nurse then followed a standardized "oropharyngeal administration of colostrum" protocol to administer the drops as depicted in Figure 1.

During administration, the nurse placed the syringe tip into the infant's mouth, along the right buccal mucosal tissue (mucous membrane that lines the inside of the cheek), and directed it posteriorly toward the oropharynx (area of the throat that is at the back of the mouth). The nurse administered 0.1 mL (approximately 7 drops) of the colostrum over a period of at least 2 minutes. Without removing the syringe from within the infant's mouth, the nurse then carefully redirected the syringe to the left buccal mucosal tissue, with the tip directed posteriorly toward the oropharynx. The nurse then slowly administered an additional 0.1 mL of the colostrum

FIGURE 1.

Extremely low birth weight infant receiving own mother's colostrum via oropharyngeal administration.

over a period of at least 2 minutes. Thus, a total of 0.2 mL, or approximately 14 drops, were administered per treatment session. This procedure was carried out every 2 hours over a period of 48 hours, beginning within 48 hours of life.

Throughout the treatment protocol, the infant's heart rate, respiratory rate, oxygen saturation, and blood pressure were monitored. The nurse was instructed to immediately stop the procedure if the infant demonstrated signs of agitation, had a significant desaturation episode with oxygen saturation less than 88%, or experienced a change in vital signs. Upon completion of the treatment protocol, the nurse recorded the date and time of colostrum administration on the intake flow sheet in the infant's electronic medical record. Tracheal aspirate and urine specimens were collected within 6 hours of completion of the treatment protocol and again 2 weeks later. All specimens were centrifuged and immediately frozen at -80°C until they were transported to a collaborating laboratory (University of Tennessee) for subsequent biochemical analysis.

RESULTS

All infants in this study completed the entire treatment protocol, each receiving a total of 24 treatments

during a period of 48 consecutive hours. The first treatment was given within 48 hours of life. Urine and tracheal aspirates were collected from each infant prior to, and at the completion of, the treatment protocol and again 2 weeks later. A total of 15 urine specimens were collected for the 5 infants; however, 1 specimen (.07%) was insufficient in volume for analysis. A total of 15 tracheal aspirates were also collected; however, 8 specimens (53%) were insufficient in volume for analysis.

For all infants in this study, oxygen saturation measures remained stable or increased slightly during each of the treatment sessions. There were no episodes of apnea, bradycardia, hypotension, or other adverse effects. All infants began to suck on the endotracheal tube during the administration of drops.

There was wide variation in concentrations of sIgA and lactoferrin in urine and tracheal aspirates among the 5 infants. The ranges of sIgA and lactoferrin concentrations for urine and tracheal aspirates are described in Table 1. Data from some samples could not be obtained because results fell above or below the limits of assay detection, on the basis of sample dilution.

DISCUSSION

This study is the first to investigate the safety and feasibility of oropharyngeal administration of own mother's colostrum drops to ELBW infants in a clinical setting. Using mother's colostrum in this manner requires a change in thinking, to view colostrum as a potential immune therapy and not simply as a feeding. As such, the oropharyngeal administration of colostrum can be an alternative to *nil per os* (nothing by mouth) status and/or a complement to trophic feeds in the first days of life for the ELBW infants.

Mothers were willing to participate in this study; 88% of the women who were approached agreed to participate in this study. In fact, many of the mothers wanted their babies to participate and receive colostrum drops because they felt that it would be beneficial. They realized that as per standard care in the NICU, an ELBW infant would not "taste" mother's milk until at least 32 weeks' corrected gestational age when peroral feeds are introduced. In this study, mothers

TABLE 1. The Ranges of Secretory Immunoglobulin A (sIgA) and Lactoferrin Concentrations for Urine and Tracheal Aspirates

Sample Source	Usable 'n'	Median	Minimum	Maximum
sIgA urine, ng/mL	14	83.8	0.8	167
sIgA tracheal aspirate, ng/mL	7	91 470	5.8	183 000
Lactoferrin urine, ng/mL	14	278.6	1.34	556
Lactoferrin tracheal aspirate, $\mu\text{g/mL}$	7	90.0	16	164

knew that their infants would taste their colostrum as early as 48 hours after birth, as opposed to 8 weeks later. This protocol was also well received by the nurses and physicians at the research site.

The pilot study demonstrated that this protocol for administering colostrum oropharyngeally was well tolerated by even the smallest and sickest ELBW infants. Oxygen saturations remained stable and no adverse events occurred. An observation of interest during the oropharyngeal administration of colostrum was that infants appeared to "taste" the colostrum, as noted by sucking on the breathing tube. This observation may have developmental implications. Mothers reacted positively to their infant's responses when "tasting" the colostrum, an observation that may have psychological benefit for these women.

The data from this study were limited for the following reasons. First, more than 50% of the tracheal aspirate samples were insufficient in quantity for analysis. This raises the question of the feasibility of using tracheal aspirates in future studies. Another limitation of this study was the lack of reference values for immune markers in ELBW infants in the first days of life, because the only existing reference values are for larger preterm or term neonates. Therefore, without a reference range for sIgA and lactoferrin, it was difficult to determine how samples should be diluted to allow data to fall within a quantifiable range. Thus, data from some specimens fell above or below the limits of the assay. These issues with sampling and measurement informed the subsequent study.

In summary, results from this pilot study showed that oropharyngeal administration of own mother's colostrum is easy, inexpensive, and well tolerated by even the smallest and sickest ELBW infants. Despite limitations, including a small sample and missing data, the information from this study, particularly in terms of subject recruitment and sample collection and analysis, provided guidance for future studies. Future research should investigate normal range references for these immune markers in ELBW infants and address the immune effects and the developmental implications of ELBW infants who receive this intervention. Of particular interest is whether this easy and inexpensive intervention may provide protection against VAP for ELBW infants in the NICU.

Current evidence suggests that many of the 130 known HMOs inhibit the adherence of bacteria to mucosal surfaces.⁵³ They may be especially important in protecting the oropharyngeal area and this may be a mechanism for protection from pneumonia in ventilator-dependent infants, when own mother's colostrum is administered oropharyngeally. Further research in this area is warranted.

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