

Oropharyngeal Colostrum Administration in Extremely Premature Infants: An RCT

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abstract

OBJECTIVE: To determine the immunologic effects of oropharyngeal colostrum administration in extremely premature infants.

METHODS: We conducted a double-blind, randomized, placebo-controlled trial involving 48 preterm infants born before 28 weeks' gestation. Subjects received 0.2 mL of their mother's colostrum or sterile water via oropharyngeal route every 3 hours for 3 days beginning at 48 to 96 hours of life. To measure concentrations of secretory immunoglobulin A, lactoferrin, and several immune substances, urine and saliva were obtained during the first 24 hours of life and at 8 and 15 days. Clinical data during hospitalization were collected.

RESULTS: Urinary levels of secretory immunoglobulin A at 1 week (71.4 vs 26.5 ng/g creatinine, $P = .04$) and 2 weeks (233.8 vs 48.3 ng/g creatinine, $P = .006$), and lactoferrin at 1 week (3.5 vs 0.9 $\mu\text{g/g}$ creatinine, $P = .01$) were significantly higher in colostrum group. Urine interleukin-1 β level was significantly lower in colostrum group at 2 weeks (55.3 vs 91.8 $\mu\text{g/g}$ creatinine, $P = .01$). Salivary transforming growth factor- β 1 (39.2 vs 69.7 $\mu\text{g/mL}$, $P = .03$) and interleukin-8 (1.2 vs 4.9 ng/mL, $P = .04$) were significantly lower at 2 weeks in colostrum group. A significant reduction in the incidence of clinical sepsis was noted in colostrum group (50% vs 92%, $P = .003$).

CONCLUSIONS: This study suggests that oropharyngeal administration of colostrum may decrease clinical sepsis, inhibit secretion of pro-inflammatory cytokines, and increase levels of circulating immune-protective factors in extremely premature infants. Larger studies to confirm these findings are warranted.



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Dr Lee conceptualized and designed the study, carried out the initial analyses, and drafted the initial manuscript; Dr Kim conceptualized and designed the study and reviewed and revised the manuscript; Drs Jung, Choi, and Shin collected data and reviewed and revised the manuscript; Drs Kim and Choi coordinated and supervised data collection and critically reviewed the manuscript; and all authors approved the final manuscript as submitted.

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WHAT'S KNOWN ON THIS SUBJECT: Immune-related bioactive proteins are highly concentrated in the colostrum of mothers who deliver preterm infants. Oropharyngeal administration was proposed as a safe and feasible alternative method of providing colostrum to immunocompromised premature infants.

WHAT THIS STUDY ADDS: Oropharyngeally administered colostrum during the first few days of life increased urinary secretory immunoglobulin A and lactoferrin, decreased urinary interleukin-1 β , reduced salivary transforming growth factor- β 1 and interleukin-8, and reduced the occurrence of clinical sepsis in extremely premature infants.

During the first few days after birth, open tight junctions of the mammary epithelium allow for paracellular transport of many bioactive immune substances from the mother's circulation into the colostrum.¹ This small volume of breast milk contains increased concentrations of secretory immunoglobulin A (sIgA), growth factors, lactoferrin, anti-inflammatory cytokines, pro-inflammatory cytokines, and other protective components, compared with mature breast milk.²⁻⁵ Several studies indicate that immunoprotective factors are more highly concentrated in the colostrum of mothers who deliver preterm infants than those who give birth at term.⁶⁻⁸ Similarly, studies suggest that closure of the tight junctions in the mammary epithelium might be delayed after preterm compared with term birth.^{9,10} However, many preterm infants cannot tolerate enteral feedings because of clinical instability and therefore do not receive maternal colostrum, possibly resulting in increased susceptibility to various infections and inflammatory conditions.

Recently oropharyngeal administration of colostrum (so-called oral immune therapy) has been advocated for preterm infants.¹¹ Oropharyngeal administration does not involve the infant's swallowing of milk. During this intervention, a small amount of colostrum is placed directly onto the oropharyngeal mucosa in the buccal cavity for absorption.¹² In theory, the abundant immune factors in colostrum interact with lymphoid tissues in the oropharynx and stimulate the immature neonatal immune system when administered via the oropharyngeal route.^{11,13} Although theoretical and preclinical support for this practice exists, there is insufficient evidence that oropharyngeal administration of colostrum is beneficial to date.¹³⁻¹⁶ We aimed to evaluate the

immunologic effects of oropharyngeal colostrum administration in extremely premature infants.

METHODS

Study Design

A randomized, double-blind, placebo-controlled, intervention trial was conducted from January 2012 to December 2013 in the NICU of Seoul National University Children's Hospital in Seoul, Korea. The local institutional review board approved the protocol. The entirety of this study was conducted in accordance with the current revisions of the Declaration of Helsinki and Good Clinical Practice guidelines.

Participants

Neonates born before 28 weeks' gestation were enrolled. Infants with congenital gastrointestinal or renal anomalies or a maternal history of substance abuse or HIV infection were excluded. After informed parental consent was obtained, regardless of twin or higher-order multiples, each neonate was randomly assigned independently to the colostrum or placebo group in a 1:1 ratio on the randomization Web site of Medical Research Collaborating Center of Seoul National University Hospital. Randomization was conducted using a computer-generated allocation sequence (block sizes of 8). Allocation was concealed from all investigators, nurses, doctors, and parents, with the exception of 1 independent research staff member, who prepared the colostrum and placebo syringes. The feeding status of each patient was decided by the attending physicians under the principle that trophic feeding should be started as soon as possible if no contraindication was found (eg, bilious gastric remain, fixed dilated bowel loop on radiograph, severe hemodynamic instability). Both groups of neonates were fed breast milk or preterm formula, whichever was prepared first. The

probiotic Duolac Baby (Cell Biotech, Seoul, Korea) was added according to the local practice protocol when the amount of each feeding was >2 mL.

Intervention

Investigators who qualified as international board-certified lactation consultants met the mothers of each enrolled neonate within 24 hours of delivery and educated them about hand-expression and electric pumping of breast milk every 2 to 3 hours. Mothers were given pre-labeled sterile milk collection bags and instructed to collect their colostrum by using a sanitary hand-expression method and then to send the colostrum to the NICU immediately for refrigeration.

Tuberculin syringes were used to administer colostrum or placebo via the oropharyngeal route. One unblinded investigator prepared 48 syringes with 0.1 mL of mother's colostrum or sterile distilled water using aseptic techniques. These syringes were then labeled and wrapped with opaque covers to maintain blinding. The syringes were placed in pre-labeled plastic cups and stored at 4°C in a specified milk refrigerator.

Beginning at 48 to 96 hours after birth, each neonate received 0.2 mL of his or her mother's colostrum or sterile water every 3 hours for 72 consecutive hours, regardless of whether the infant was being fed enterally. At each session, 2 pre-filled syringes with 0.1 mL of colostrum or sterile water were warmed in the infant's incubator for 5 minutes. One syringe was placed on the patient's right or left buccal mucosa, and the colostrum or placebo drops were administered toward the posterior oropharynx for at least 10 seconds (Fig 1 and Supplemental Video). The same process was repeated on the opposite site, as described in a previous study.¹⁴ Heart rate (HR), respiratory rate (RR), blood pressure, and pulse oxygen saturation (SpO₂)

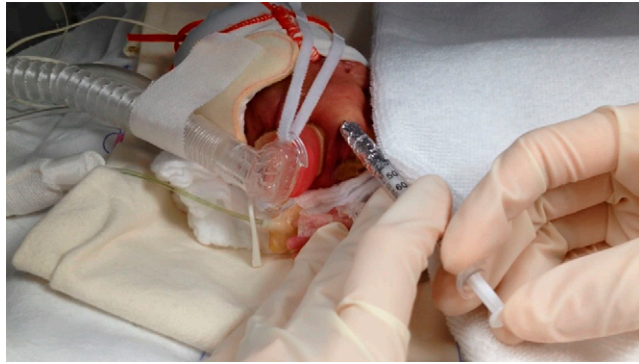


FIGURE 1
A still image at 00:05 from the sample video demonstrating oropharyngeal administration of colostrum (corresponding Supplemental Video).

were recorded immediately before and after every intervention session. Colostrum or sterile water was not administered during surgery under general anesthesia. A session was discontinued if any of the following issues developed: requirement of an increase in fraction of inspiratory oxygen >0.1 to maintain a $SpO_2 >85\%$, bradycardia (HR <100 /minute) or tachycardia (HR >200 /minute), and tachypnea (RR >80 /minute).

Assessments and Monitoring

To evaluate the salivary production of bioactive proteins after activation of oropharyngeal mucosa-associated lymphoid tissue (MALT) and the urinary excretion of bioactive proteins after systemic circulation, we measured the concentrations of immunologic factors in saliva and urine. We also looked at the incidence of late-onset sepsis and other inflammatory medical comorbidities of prematurity, such as necrotizing enterocolitis (NEC), bronchopulmonary dysplasia, ventilator-associated pneumonia (VAP), retinopathy of prematurity, intraventricular hemorrhage, clinical or proven sepsis, time to reach full feeding (100 mL/kg/day), hospitalization duration, and mortality. We defined VAP as clinical signs of pneumonia combined with pneumonic infiltration on ≥ 2 serial chest radiographs in patients receiving mechanical ventilation for >48 hours. Clinical signs of

pneumonia included worsening gas exchange, increased oxygen requirements, increased ventilator demand, and ≥ 1 clinical symptoms (new onset of purulent sputum, temperature instability, leukopenia/leukocytosis with left shift, apnea/tachypnea, or bradycardia/tachycardia). Clinical sepsis was defined as clinical signs of infection accompanied by concurrent antibiotic treatment of >3 days. Clinical signs of infection included all 3 of the following categories and at least 1 sign in each of the 3 categories: general signs (fever; apnea/tachypnea, respiratory distress, positive fluid balance), laboratory results (leukopenia/leukocytosis, increased C-reactive protein), and hemodynamic alterations (hypotension, tachycardia, altered skin perfusion, decreased urine output, increased base deficit).¹⁷ Proven sepsis was defined as bacterial growth in at least 1 blood culture and fulfillment of clinical sepsis.

Vital signs were monitored throughout the study, and any occurrence of adverse events was recorded. Clinical data from each patient's hospitalization were collected at discharge from the NICU.

An independent data and safety-monitoring board supervised the investigation and reviewed the data from the first 3 patients and after completion of the study. The board had access to all data, and none of their analyses resulted in

modifications or termination of this study.

Specimen Collection and Assays

To measure the concentrations of immunologic factors, urine and saliva were collected during the first 24 hours and at 8 and 15 days of life. Urine was obtained by using a sterile attachable urine bag for neonates. Unstimulated whole saliva was collected in a sterile container using weak suction. All specimens were centrifuged, aliquoted, and stored at -70°C until biochemical analysis.

The concentrations of sIgA, lactoferrin, transforming growth factor (TGF)- $\beta 1$, and interleukin (IL)-10 from the urine and saliva specimens were measured using enzyme-linked immunosorbent assay kits (sIgA: USCN Life Science, Wuhan, China; lactoferrin: EMD Millipore, Billerica, MA; TGF- $\beta 1$ and IL-10: R&D Systems, Minneapolis, MN) according to the manufacturer's protocols. Epidermal growth factor (EGF), tumor necrosis factor- α , interferon- γ , IL-1 β , IL-2, IL-4, IL-6, and IL-8 were measured using luminex fluorescent bead human cytokine immunoassays (MILLIPLEX MAP, Millipore).

Sample Size Calculation and Statistical Analysis

On the basis of results from a previous study,¹⁸ we assumed a mean difference in urinary sIgA of 29.75 $\mu\text{g/mL}$ between the 2 groups, and the SDs were 41.55 $\mu\text{g/mL}$ for the colostrum group and 9.75 $\mu\text{g/mL}$ for the placebo group. Given an asymptotic relative efficacy on the 80% power and a 5% significance level with the independent *t* test, the required sample size for the Mann-Whitney *U* test was calculated as 21 infants for each group. Therefore, assuming a 10% dropout rate, we estimated that 48 subjects were needed.

Analysis of the clinical data was performed on an intention-to-treat basis, and the biochemical data of specimens from subjects who

completed the protocol were analyzed. Statistical analyses were performed by using SPSS version 21.0 (SPSS, Chicago, IL). Mann-Whitney *U* tests or Fisher's exact tests and regression analyses were used for group comparisons.

RESULTS

Of 75 extremely premature infants born <28 weeks' gestation from January 2012 to December 2013, 48 were included and randomly assigned to the placebo or colostrum group (Fig 2). Two of 24 infants were excluded from the placebo group because of death before study initiation, and 1 infant was withdrawn from the study due to parental wishes. Three infants were excluded from the colostrum group: 2 due to the absence of colostrum until 96 hours after birth, and 1 due to death before study initiation. Forty-two of 48 infants completed the protocol. The median number of received doses was 24 (interquartile range [IQR]: 23–24) in both groups. Fifteen and sixteen infants received all 24 doses for the placebo and colostrum group, respectively. The median gestational age of the population was 26⁺⁵ weeks (range: 23⁺¹–27⁺⁶ weeks), and the median birth weight was 815 g (range: 400–1450 g).

Table 1 shows no differences in the baseline characteristics of the study population or in the number that received formula before starting the protocol, during 3 days of intervention, and during a 2-week period after birth. Approximately half of the enrolled infants were nil per os (NPO) before the study, and 18 (37.5%) infants did not start enteral feeding during the first week of life.

Figure 3 demonstrates urine levels of immune substances based on creatinine concentrations. The urinary sIgA level at 1 week was significantly increased in the colostrum group (71.4 vs 26.5 ng/g creatinine, *P* = .04), and remained

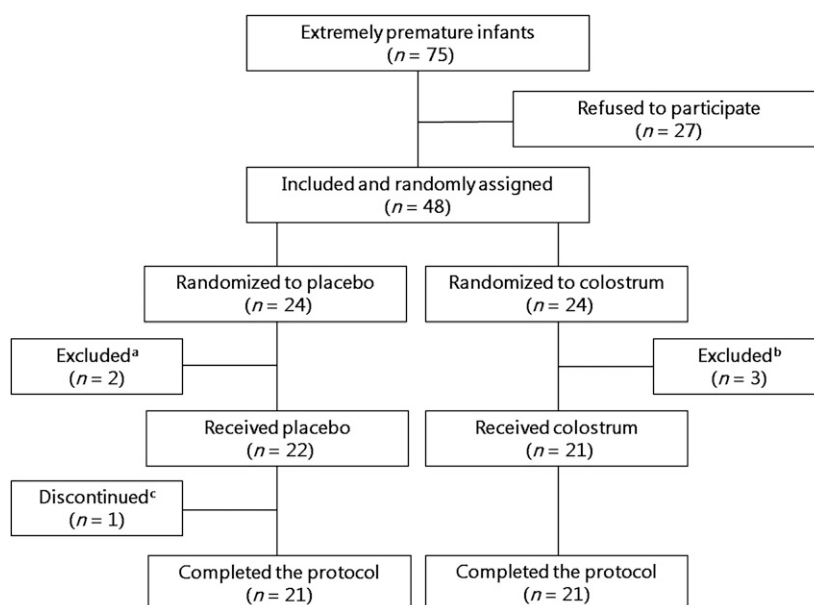


FIGURE 2

Study profile. ^aExcluded from the placebo group because of death before intervention (*n* = 2). ^bExcluded from the colostrum group because of the absence of maternal colostrum (*n* = 2) or death before intervention (*n* = 1). ^cOne infant discontinued the study based on the parents' withdrawal of consent.

elevated at 2 weeks (233.8 vs 48.3 ng/g creatinine, *P* = .006). Urinary lactoferrin level was also significantly

increased at 1 week in the colostrum group (3.5 vs 0.9 μg/g creatinine, *P* = .01). Among the interleukins, urinary

TABLE 1 Patient Demographics and Baseline Characteristics

	Placebo Group (N = 24)	Colostrum Group (N = 24)
Gestational age, wk	26 ⁺⁵ (24 ⁺³ –27 ⁺¹)	26 ⁺⁵ (24 ⁺² –27 ⁺⁴)
Birth wt, g	815 (610–1003)	830 (701–993)
Male/female ratio	10:14 (42:58)	12:12 (50:50)
Apgar score at 1 min	3 (2–5)	3 (2–5)
Apgar score at 5 min	7 (5–7)	6 (5–7)
Multiple gestation	16 (67)	22 (92)
Vaginal delivery	11 (46)	11 (46)
Antenatal steroid use	20 (83)	21 (88)
Histologic chorioamnionitis ^a	14 (58)	8 (33)
Surfactant use	20 (83)	17 (71)
Feeding before the protocol	12 (50)	11 (46)
Feeding during the protocol	15 (63)	15 (63)
Breast milk	5	4
Preterm formula	9	8
Mixed	1	3
None	9	9
Feeding for 2 wk after birth	20 (83)	20 (83)
Breast milk	5	6
Preterm formula	11	11
Mixed	4	3
None	4	4
Mechanical ventilation at randomization	18 (75)	14 (58)
≥1 transfusion during 2 wk after birth	18 (75)	17 (71)
Prostaglandin inhibitor use	11 (46)	11 (46)
Postnatal steroid use	8 (33)	12 (50)
H ₂ blocker use	7 (29)	8 (33)
Probiotic use	18 (75)	19 (79)

Values are median (IQR) or number (%).

^a Presence of acute inflammatory changes on fetal membranes (subchorion, chorion, amnion, or umbilicus).

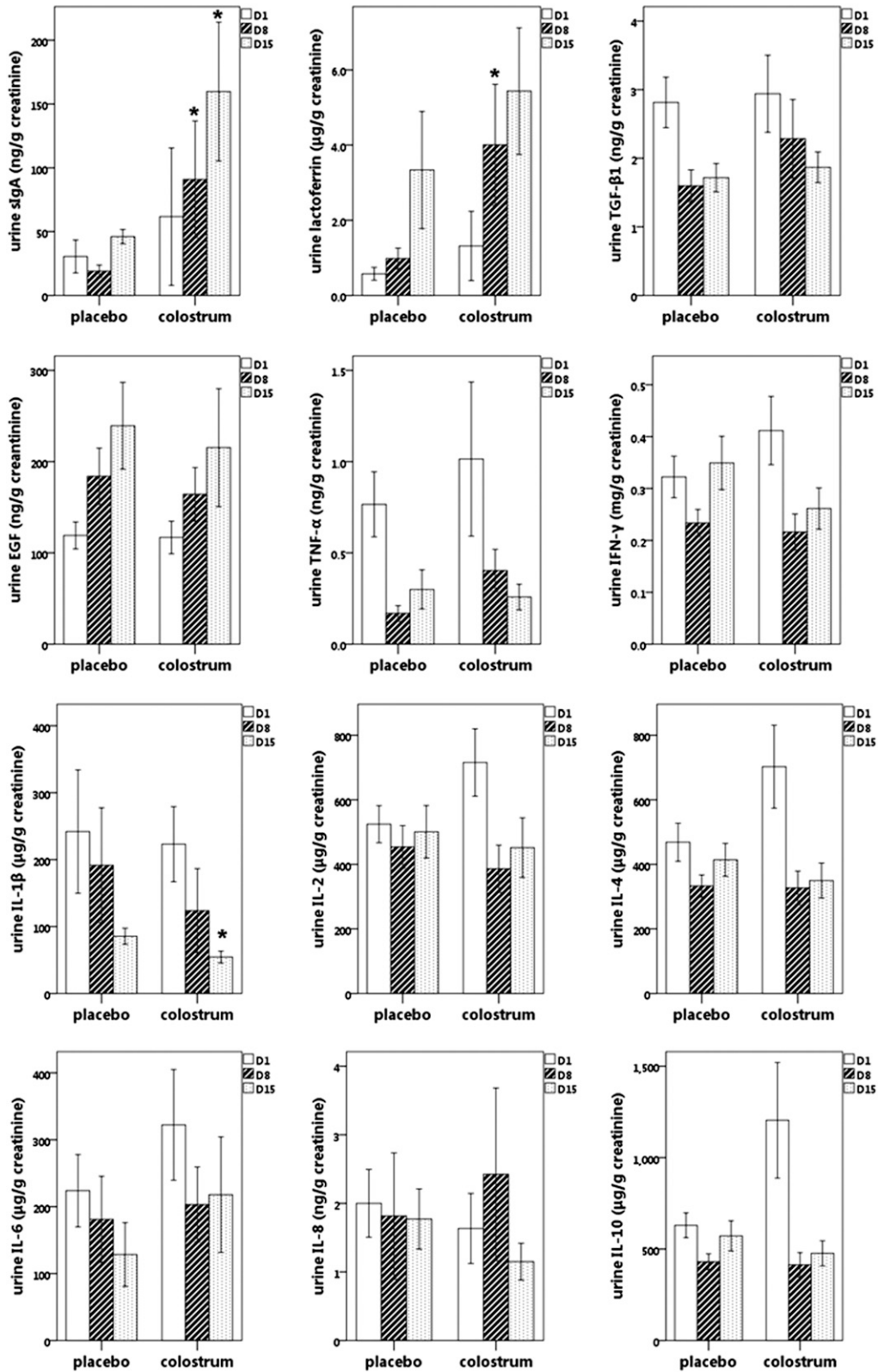


FIGURE 3

Urinary levels of immune substances based on creatinine concentrations on days 1, 8, and 15, from 42 infants who completed the protocol. Each error bar represents 1 SE. Mann-Whitney *U* tests were used for group comparisons, * *P* < .05 versus placebo group.

IL-1 β level was significantly reduced at 2 weeks in the colostrum group (55.3 vs 91.8 $\mu\text{g/g}$ creatinine, $P = .01$). Regarding the immune substances in saliva (Fig 4), the concentrations of sIgA (5.4 vs 2.1 $\mu\text{g/mL}$, $P = .02$) and EGF (464.3 vs 258.4 pg/mL , $P = .04$) were significantly increased at 1 week in the colostrum group but were decreased to levels similar to that of the placebo group at 2 weeks. Salivary TGF- β 1 (39.2 vs 69.7 $\mu\text{g/mL}$, $P = .03$) and IL-8 (1.2 vs 4.9 ng/mL , $P = .04$) were significantly reduced in the colostrum group at 2 weeks compared with the placebo group.

The colostrum group had less clinical sepsis (50% vs 92%, $P = .003$) and shorter total antibiotic duration (6 [IQR: 3.8–8.5] vs 9.5 [IQR: 7–19] days, $P = .014$), but no difference in culture-proven sepsis (46% vs 58%, $P = .56$) (Table 2). The significant effect of colostrum administration on clinical sepsis was also validated in a regression analysis with all possible confounders, including mechanical ventilation, H2 blocker use, probiotic use, postnatal steroid use, feeding status and types (exp[B] = 67.3, 95% confidence interval: 3.8–1186.9, $P = .004$). No differences in NEC, bronchopulmonary dysplasia, VAP, grade ≥ 3 intraventricular hemorrhage, retinopathy of prematurity that required laser surgery, time to reach full enteral feeding, hospitalization duration, or mortality were noted. Blood pressure, HR, RR, and SpO $_2$ remained stable during each administration session. No episodes of agitation, aspiration events, bradycardia or tachycardia, hypotension, or other acute adverse events were noted in any of the infants during the interventions.

DISCUSSION

To the best of our knowledge, this is the first double-blind, randomized, placebo-controlled trial to provide immunologic and clinical evidence regarding the advantages of

oropharyngeal colostrum administration in extremely premature infants. A prospective study of 15 extremely low birth weight infants demonstrated that the time to reach full enteral feeding was reduced,¹⁵ and 1 retrospective study reported that oropharyngeal administration of colostrum resulted in starting feeding earlier and reaching birth weight sooner.¹⁶ However, no published data have illustrated the clinical advantages of oropharyngeal colostrum administration in relation to the immune system.

Colostrum contains higher concentrations of immunoprotective agents compared with mature human milk,⁵ and these agents are believed to compensate for the delayed immune system development of premature infants by conducting immunomodulatory reactions at mucosal and systemic sites.¹² Despite the theoretical advantages, providing maternal colostrum to extremely premature infants in the early postnatal period presents a variety of challenges. During this time, enteral feedings are frequently disturbed by immature gastrointestinal function and comorbidities that compromise splanchnic perfusion, such as patent ductus arteriosus, umbilical catheterization, or hypotension.¹⁹

The oropharyngeal mucosal route was recently proposed as a solution for providing maternal colostrum to the sickest infants during the early postnatal period.^{11–14,16} Rodriguez et al¹² described the expected mechanisms through which cytokines and other immunologic factors in colostrum stimulate the immature neonatal immune system via lymphoid tissues in the oropharynx and gut, resulting in the development of a protective mucosal immune barrier.

We observed that urinary excretion of sIgA and lactoferrin was significantly increased by oropharyngeal colostrum administration in

extremely premature infants (Fig 3). This result could be interpreted mainly as excretion after their passage through the systemic circulation after being absorbed by the oral or gastrointestinal mucosa. The half-lives of sIgA and lactoferrin are 3 to 6 days, and their uptake from breast milk via neonatal gut mucosa with subsequent excretion of their intact maternal forms in the urine were well demonstrated in previous studies.^{7,20–22} This exceptional mucosal absorption might be expected to occur only in preterm infants, particularly before “gut closure,” and our findings support this hypothesis.^{23,24} On the other hand, in addition to mucosal absorption, the result of continued increase of urinary sIgA at 2 weeks of age might be reflective that oropharyngeal stimulation by colostrum enhances endogenous production and/or excretion of sIgA.

Interestingly, the urinary excretion of IL-1 β was significantly decreased by oropharyngeal administration of colostrum (Fig 3). Cytokines have short half-lives of several hours and are known to be involved in mucosal immunity mainly by binding to cellular receptors.² Because the passive uptake of cytokines by mucosal barriers during the neonatal period has not been elucidated, urinary levels of cytokines might reflect endogenous production by immune systems. Contrary to evidence suggesting that the production of several cytokines by neonatal T cells are either slightly (tumor necrosis factor- α)²⁵ or markedly reduced (IL-4, IL-6, IL-8, IL-10, and interferon- γ),^{26–29} IL-1 β is known to be overproduced in preterm neonates and to be involved in excessive intestinal and systemic inflammation.³⁰ It has been noted that IL-1 β initiates inflammatory cascades and enhances the expression of a powerful chemokine, IL-8, in immature intestinal cells.^{30–32} In this regard, our data suggest the hypothesis that oropharyngeal

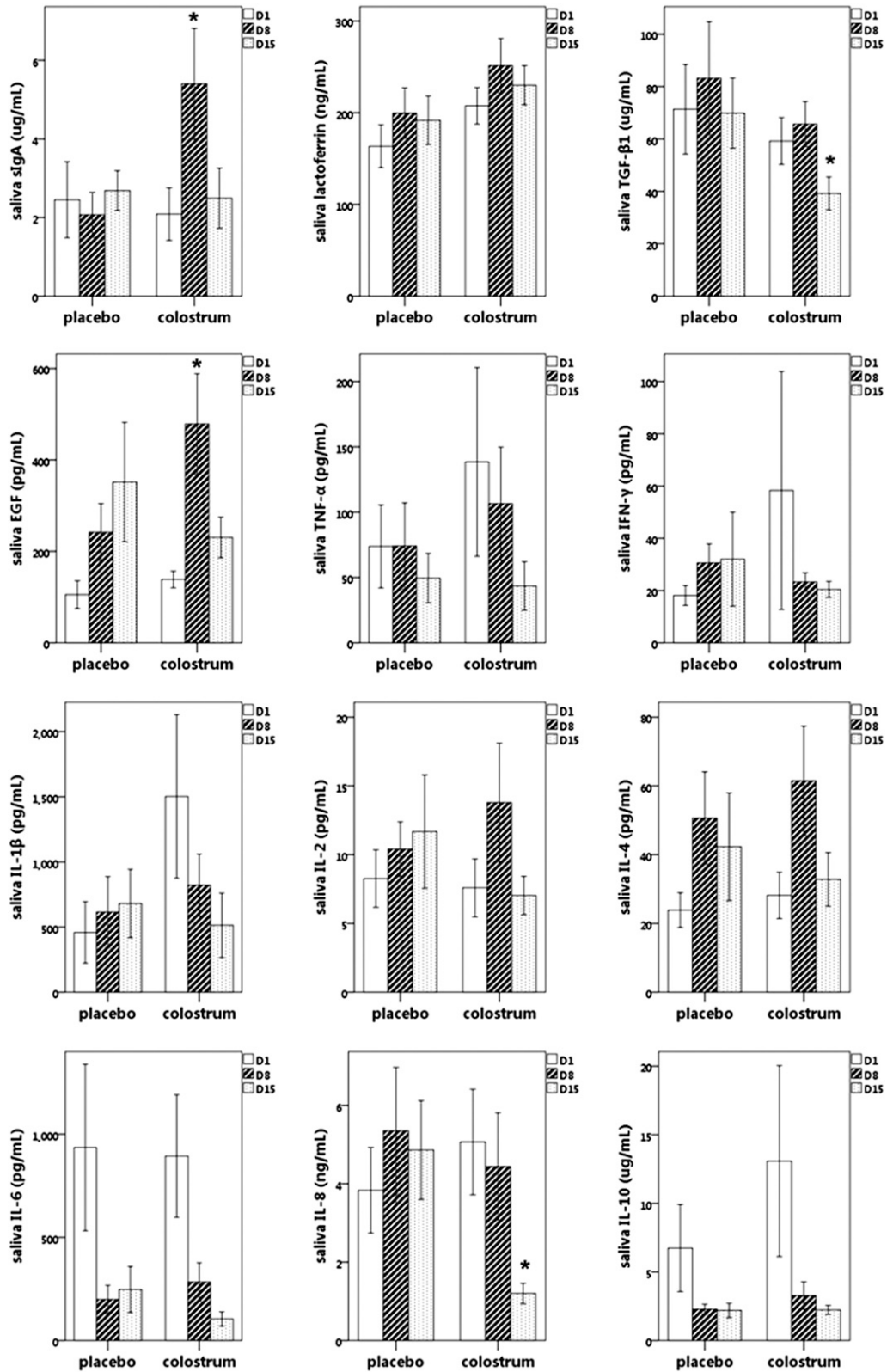


FIGURE 4

Salivary levels of immune substances on days 1, 8, and 15 from 42 infants who completed the protocol. Each error bar represents 1 SE. Mann-Whitney *U* tests were used for group comparisons, * $P < .05$ versus placebo group.

colostrum administration downregulates production and/or excretion of IL-1 β , which in turn decreases the production of IL-8.

Significantly decreased salivary levels of TGF- β 1 and IL-8 in the colostrum group could be interpreted in a similar manner (Fig 4). TGF- β 1 is the predominant isoform of TGF- β produced by immune cells within the mucosal lamina propria, and the salivary secretion of TGF- β 1 is known to play a key role in active inordinate mucosal inflammation.^{33–35} IL-8, an important chemotactic factor for neutrophils, is a known initiator of excessive intestinal inflammatory responses in preterm NEC models.^{2,30,31,36} Evidence suggests that human milk factors suppress the induction of IL-8 expression in cultured mucosal epithelial cells; this suppression is more pronounced in immature cells.³⁷

Abrupt increases in salivary sIgA and EGF concentrations in the colostrum group at 1 week were potentially influenced by orally administered colostrum (Fig 4). Because the protocol had been performed for 3 days, from the second to fourth postnatal day, a large amount of sIgA and EGF included in colostrum could remain in the oral cavity and might be subsequently collected with saliva at 1 week.

Our results demonstrate a significant reduction in the incidence of clinical but not proven sepsis in the colostrum group (Table 2). Because we confined proven sepsis to bacterial growth in any blood culture, viral infection or other systemic inflammatory responses precluded a diagnosis of proven sepsis. However, the results of several studies support that abundant immunomodulatory molecules in colostrum seem to have the capacity

TABLE 2 Clinical Outcomes at the Time of Discharge

	Placebo Group (N = 24)	Colostrum Group (N = 24)	P ^a
NEC, Bell stage \geq 2	6 (25)	4 (17)	.72
Bronchopulmonary dysplasia ^b	14 (58)	15 (63)	.58
Ventilator-associated pneumonia	8 (33)	3 (12.5)	.17
Proven sepsis	14 (58)	11 (46)	.56
Clinical sepsis	22 (92)	12 (50)	.003
Total antibiotic days	9.5 (7–19)	6 (3.8–8.5)	.014
Intraventricular hemorrhage, grade \geq 3	3 (12.5)	4 (16.7)	.34
Laser surgery for ROP	7 (29)	11 (46)	.26
Postnatal days to reach full feeding	17 (14.3–25.8)	20 (13–27)	.86
Hospital stay, d	81.5 (56.5–99)	89 (69.3–109.8)	.44
Death	6 (25)	3 (12.5)	.46

Values are median (IQR) or number (%). ROP, retinopathy of prematurity.

^a Fisher's exact test or Mann-Whitney *U* test was used for analysis.

^b Defined as requiring supplemental oxygen at 36 wk.

to decrease infection caused by bacteria, viruses, and possibly fungi without the use of inflammatory mechanisms.^{32,36,38,39} If the main immunologic effect of colostrum is to suppress mucosal and systemic inflammatory responses, the significant reduction of clinical sepsis by colostrum might reflect its capacity to downregulate immature, excessively exaggerated inflammatory responses to a variety of stimuli in newborns. In this sense, oropharyngeal administration of colostrum might be beneficial in preventing NEC or VAP. However, the number of infants in our study was too small to draw any conclusion about decreased risk of NEC or VAP.

Our trial had several other limitations. There was a high incidence of clinical and proven sepsis. The incidence of NEC was also relatively high, despite probiotic use. Furthermore, breastfeeding rates during postnatal 2 weeks were as low as ~35%; however, there was no difference in the pattern of feeding or other factors that could affect the immune response, including chorioamnionitis, transfusion, and the use of postnatal steroids, H2 blockers, and probiotics, between the 2 groups.

Although this small study cannot draw a conclusive statement about the clinical benefit of colostrum, it provides evidence suggesting that oropharyngeal administration of colostrum during the first few days of life can potentially enhance immune function in the sickest premature infants. Additionally, our findings suggest the possible usefulness of colostrum as an oropharyngeal immune-boosting agent to prevent sepsis and excessive mucosal inflammation in the preterm population. Larger-scale studies are needed to prove these effects.

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Oropharyngeal Colostrum Administration in Extremely Premature Infants: An RCT

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