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Original Article

Greater Mortality and Morbidity in Extremely Preterm Infants Fed a Diet Containing Cow Milk Protein Products

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Abstract

Background: Provision of human milk has important implications for the health and outcomes of extremely preterm (EP) infants. This study evaluated the effects of an exclusive human milk diet on the health of EP infants during their stay in the neonatal intensive care unit.
Subjects and Methods: EP infants <1,250 g birth weight received a diet consisting of either human milk fortified with a human milk protein-based fortifier (HM) ($n = 167$) or a diet containing variable amounts of milk containing cow milk-based protein (CM) ($n = 93$). Principal outcomes were mortality, necrotizing enterocolitis (NEC), growth, and duration of parenteral nutrition (PN).
Results: Mortality (2% versus 8%, $p = 0.004$) and NEC (5% versus 17%, $p = 0.002$) differed significantly between the HM and CM groups, respectively. For every 10% increase in the volume of milk containing CM, the risk of sepsis increased by 17.9% ($p < 0.001$). Growth rates were similar between groups. The duration of PN was 8 days less in the subgroup of infants receiving a diet containing <10% CM versus ≥10% CM ($p < 0.02$).
Conclusions: An exclusive human milk diet, devoid of CM-containing products, was associated with lower mortality and morbidity in EP infants without compromising growth and should be considered as an approach to nutritional care of these infants.

Introduction

Human milk provides important advantages to health outcomes of extremely preterm (EP) infants. The emergence of donor human milk to support policies recommending its use has increased rapidly in the United States and throughout the world.[1,2]

However, the use of human milk as a primary nutrition source in EP infants requires that the milk be fortified with nutrients, especially protein and micronutrients, to achieve adequate growth, body composition, and micronutrient status.[3] Currently, most EP infants receive some cow milk-based protein (CM), either as their primary diet via infant formula or as a multinutrient fortifier directly added to a human milk-based diet. A human milk protein-based fortifier (HM) for human milk (Prolact+ H^2MF; Prolacta Bioscience, City of Industry, CA) has been developed. The effect of an exclusive human milk diet on key outcomes in EP infants has been reported.[4,5] The current report combines the original individual subject results from these studies to provide a comprehensive evaluation of the risk associated with the exposure of EP infants to cow milk protein.

Subjects and Methods

An exclusively human milk diet was evaluated in two clinical trials of EP infants, defined as those born at <1,250 g birth weight.[4,5] Both trials used the same approach to feeding and evaluation of outcomes. The first of these trials,[4] concluded in 2008, involved infants at 12 centers, all but one of these in the United States, whose mothers had committed to providing them with their milk during the neonatal intensive care unit stay. In brief, infants were randomly assigned to either an exclusive human milk diet (mother's own milk or donor human milk) that included a human milk-based human milk fortifier or to a routine approach in which mother's own

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This study is registered at ClinicalTrials.gov with clinical trial registration number NCT00506584.

milk was fortified with a cow milk-based fortifier containing cow milk protein. In that group, if mother's own milk was not available, a cow milk-based infant formula product was used to supplement the mother's own milk, but donor human milk was not provided.

The second trial[5] was completed in 2010 and included infants whose mothers, for any reason, were unable or unwilling to provide their milk during the neonatal intensive care unit stay. In that trial, infants were assigned randomly to receive either an all human milk-based diet using donor human milk and human milk-based human milk fortifier (Prolact+) or cow milk-based formula products. The protocol, other than feeding assignments and blinding, and study end points were the same as for the first trial.

Taken together, these two trials provided a cohort of 260 EP infants who received a diet that ranged from 100% cow milk to 100% human milk. The preceding articles[4,5] reported the results of each of the trials as analyzed by treatment group assignment. The results being reported here represent the post hoc analysis of these two randomized clinical trials and are, in a sense, a meta-analysis of those sets of data. Of course, the individual data points are available, making this a more detailed evaluation than in the typical meta-analysis. Here, the complete cohort data were combined and analyzed in two ways. First, the results of the two studies were combined to investigate differences between infants receiving any cow milk-based nutrition in their diet and infants receiving only a 100% human milk diet (no cow milk-based nutrition). Second, taking advantage of the larger number of patients and broader range of exposures to cow milk-based products, the combined results were analyzed to determine if any of the observed differences in outcome was attributed to the proportion of human milk or cow milk-based product received during the study period.

All analyses were based on the intent-to-treat designation, except as specifically noted. Infants in the two trials who were assigned to only human milk protein are designated as Group HM (human milk fortified with a human milk protein-based fortifier), and those who were assigned to receive any cow milk protein, either as human milk fortifier or infant formula, are designated as Group CM.

Descriptive statistics for quantitative data were used to summarize the characteristics of the two groups on a univariate basis. These included mean ± SD for nonskewed data and median ± interquartile range for skewed data (e.g., milk intake). The groups were compared using the nonparametric Wilcoxon rank-sum test. The chi-squared test for homogeneity or Fisher's exact test (if the expected frequencies in any given cell were less than 5) was used to compare the proportions in the two groups from a univariate perspective. Ninety-five percent confidence intervals were calculated using large sample (normal distribution) methods for the difference in outcomes between the CM and HM groups, including necrotizing enterocolitis (NEC)/surgical NEC and sepsis risk, and growth velocities. For mortality, an exact calculation of the 95% confidence interval for the difference in risk was based on the Blythe–Still–Casella method.[6] For parenteral nutrition (PN) days, because of the censoring in this variable, the method of Kaplan and Meier[7] was used to estimate the distribution of these events, and the formula of Greenwood[8] was used to help in the determination of the 95% confidence interval for the difference in medians.

Multivariate binary logistic regression was used to evaluate key end points, such as the occurrence of NEC, NEC surgery, and sepsis. These models were used to evaluate the effect of the amount of the diet from cow milk sources using odds ratios and 95% confidence intervals. The significance of the regression coefficients was assessed by the usual Wald's *Z*-statistic. In addition, adjustments for key covariates were incorporated into the models. For NEC and surgical NEC, we used Apgar scores, gestational age, use of antenatal steroids,

Table 1. Patient Characteristics

Parameter	*Group CM* (n = *93*)	*Group HM* (n = *167*)	p *value*
Gender (male)	47 (51%)	69 (41%)	0.15[a]
Completed study	70 (75%)	134 (80%)	0.35[a]
Transferred to another hospital	16 (17%)	21 (13%)	0.10[a]
Withdrew consent	0	2 (1%)	0.54[b]
Race (black)	21 (23%)	50 (30%)	0.22[a]
BPD	28 (30%)	50 (30%)	0.98[a]
SGA	10 (11%)	15 (9%)	0.36[a]
Prenatal steroids	71 (82%)	128 (81%)	0.83[a]
Postnatal steroids	23 (30%)	39 (28%)	0.71[a]
Apgar score < 6	11 (12%)	13 (8%)	0.28[a]
Mechanical ventilation at study entry	53 (76%)	105 (76%)	0.95[a]
Birth weight (mean ± SD)	938 ± 200	939 ± 192	0.97[c]
GA (mean ± SD)	27 ± 2	27 ± 2	0.85[c]
Volume (mL) [median (25th, 75th percentile)]			
Mother's milk	2,102 (1, 7,174)	1943 (127, 6,960)	0.96[c]
Donor human milk	0	883 (0, 4,581)	
Formula	2,109 (54, 5,764)		
Total milk intake	7,190 (4,402, 10,008)	8,169 (5,331, 11,016)	0.13[c]

[a]By chi-squared test.
[b]By Fisher's exact test.
[c]By Wilcoxon rank-sum test.
BPD, bronchopulmonary dysplasia; CM, milk containing cow milk-based protein; GA, gestational age; HM, human milk fortified with a human milk protein-based fortifier; SGA, small for gestational age.

Table 2. Outcome of Study Infants

	Group CM (n = *93*)	*Group HM* (n = *167*)	p *value*	*95% CI (difference)*
Mortality	7/93 (8%)	3/167 (2%)	0.04	0.3% to 13%
NEC	16/93 (17%)	9/167 (5%)	0.002	2.4% to 21.3%
Surgical NEC	11/93 (12%)	2/167 (1%)	0.0003	4.4% to 18.9%
Sepsis	32/93 (34%)	50/167 (30%)	0.46	−7.1% to 16.6%
TPN (median days)[a]	25	23	0.26	−2 to 5
Weight (g/kg/day)	13.6 ± 4.1	14.9 ± 7.2	0.11	−0.1 to 2.7
Length (cm/week)	0.89 ± 0.45	0.97 ± 0.35	0.12	−0.02 to 0.19
FOC (cm/week)	0.73 ± 0.23	0.77 ± 0.22	0.10	−0.01 to 0.11

[a]From the Kaplan and Meier[7] estimate.

CI, confidence interval; CM, milk containing cow milk-based protein; FOC, fronto-occipital circumference; NEC, necrotizing enterocolitis; HM, human milk fortified with a human milk protein-based fortifier; TPN, total parenteral nutrition.

African American race, and presence of bronchopulmonary dysplasia because each of these has been previously identified as potential risk factors for NEC.[9–11] For sepsis, the adjustment covariates were birth weight, days of PN, days on a central line, days on a ventilator, and Apgar score. Again, these are known risk factors for the development of sepsis in this population.[12–14] In all cases a p value of 5% or less was set as the definition of statistical significance.

Informed written consent was obtained from parents separately for each trial after review and approval of the Institutional Review Board for Human Subject Research at each site.

Results

Characteristics of the study subjects and study outcomes are given in Tables 1 and 2. The patient populations were not significantly different with respect to characteristics and various clinical outcomes. However, most importantly, significantly lower mortality ($p = 0.04$), risk of NEC ($p = 0.002$), and NEC requiring surgery within 2 weeks of diagnosis ($p = 0.0003$) were seen in Group HM versus Group CM.

The incidence of NEC, NEC requiring surgery, and sepsis were evaluated using a multivariate logistic model to evaluate whether the percentage of the diet containing cow milk protein had an impact on those outcomes. Potentially mitigating covariates were included in the model. For the two NEC models these included Apgar score, gestational age, receipt of antenatal steroids, black race, and presence of bronchopulmonary dysplasia. The results of these models are given in Table 3. The percentage of diet containing cow milk was a significant predictor of NEC ($p = 0.047$) and NEC surgery ($p = 0.01$). The other covariates, although considered to be risk factors for NEC and NEC surgery, did not mitigate this effect. The odds ratios for cow milk percentage can be interpreted as follows: for each 10% (an incremental value selected to allow ease of interpretation of the odds ratios from the regression models) increase in the intake of other than an exclusive human milk diet, the risk of NEC increases by 11.8% (95% confidence interval, 0.2–24.8%), and the risk of surgical NEC increases by 21% (95% confidence interval, 4.2–39.6%). The multivariate model for sepsis risk as a function of the percentage of diet from cow milk is given in Table 4. The key potentially mitigating covariates in this model included Apgar score, birth weight, PN duration, central line days, and ventilation

Table 3. Multivariate Logistic Models for Necrotizing Enterocolitis (NEC) and Surgical NEC as a Function of the Percentage of the Diet Containing Cow Milk Protein

Parameter	*Odds ratio (95% confidence interval)*	p *value*
All NEC[a]		
% dietary volume from cow milk	1.01 (1.00, 1.02)	0.047
Apgar score	1.12 (0.82, 1.52)	0.48
GA	0.86 (0.68, 1.10)	0.22
Antenatal steroids	1.10 (0.34, 3.55)	0.87
Black race	0.76 (0.29, 2.03)	0.59
BPD	0.84 (0.30, 2.33)	0.74
Surgical NEC[b]		
% dietary volume from cow milk	1.02 (1.004, 1.03)	0.01
Apgar score	1.29 (0.80, 2.09)	0.30
GA	0.79 (0.56, 1.12)	0.19
Antenatal steroids	0.82 (0.16, 4.28)	0.81
Black race	1.20 (0.27, 5.31)	0.81
BPD	0.98 (0.24, 3.92)	0.97

[a]Fit of the regression model: pseudo-$R^2 = 0.039$, area under the receiver operator characteristic curve = 0.65.

[b]Fit of the regression model: pseudo-$R^2 = 0.087$, area under the receiver operator characteristic curve = 0.70.

BPD, bronchopulmonary dysplasia; GA, gestational age.

Table 4. Multivariate Logistic Models for Sepsis as a Function of the Percentage of the Diet Containing Cow Milk Protein

Parameter	*Odds ratio (95% confidence interval)*	p *value*
% dietary volume from cow's milk	1.02 (1.01, 1.025)	0.00006
Birth weight	0.999 (0.997, 1.001)	0.21
TPN days	1.04 (1.01, 1.06)	0.009
Central line days	1.02 (0.996, 1.04)	0.10
Ventilation days	0.99 (0.98, 1.005)	0.19
Apgar score	1.03 (0.85, 1.24)	0.80

Fit of the regression model: pseudo-$R^2 = 0.15$, area under the receiver operator characteristic curve = 0.77.

TPN, total parenteral nutrition.

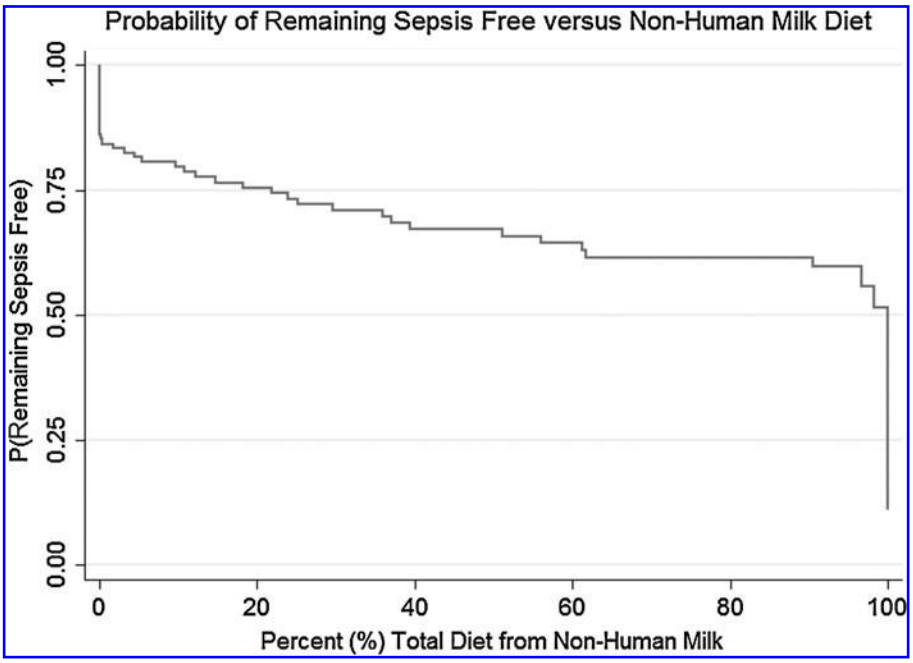

FIG. 1. Inverse empirical cumulative distribution of sepsis as a function of the amount of nonhuman milk in the diet.

days. The percentage of diet from cow milk was significantly associated with the risk of sepsis ($p = 0.00006$). For each 10% increase in the intake of other than an exclusive human milk diet, there was a 17.9% increase in risk in sepsis (95% confidence interval, 8.8–27.8%). The overall relationship between the risk of sepsis and amount of nonhuman milk is shown in Figure 1 as an inverse empirical cumulative distribution function.

We did not find any overall effect on length of time for PN. However, in a subgroup analysis of infants receiving <10% (a reasonably small number) of their diet as formula ($n = 182$), the number of PN days was lower than in the subgroup receiving ≥10% of the diet as formula ($n = 78$): 21 versus 29 days ($p = 0.02$). The incidence of sepsis also was significantly lower in the <10% formula group (24% versus 49% [$p < 0.0001$]), as was the percentage of NEC (6% versus 18% [$p < 0.001$]) and NEC surgery (3% versus 10% [$p = 0.01$]).

Discussion

We have shown that provision of an exclusively human milk diet during the early postnatal period, a diet devoid of cow milk protein, is associated with lower risks of death, NEC, NEC requiring surgery, and sepsis in EP infants. This information is necessary to calculate a cost:benefit ratio so that healthcare teams can support the optimization of nutritional outcomes in EP infants. The cost of major complications of extreme prematurity, such as sepsis and NEC, is high, typically more than $250,000 for each case of NEC that requires surgery.[15] Lifetime costs are likely much higher because of the increased risk of long-term neurodevelopmental problems in infants who have had NEC requiring surgery.[16] These outcomes are the major drivers of costs and long-term morbidity in this population, and their avoidance is a principal goal of contemporary neonatal care. A recent analysis confirmed that human milk costs are minimal compared with the costs of the major morbidities in very low birth weight infants.[17]

The results of this analysis indicate a significant effect on overall mortality as well as sepsis that was not identified in the individual studies previously reported.[4,5] The combined studies demonstrate a dose-related effect of nonhuman milk intake in increasing negative patient outcomes. Furthermore, by including infants with a range of cow milk protein intake, we have shown a significant benefit to using very minimal cow milk protein on the duration of PN, a measure of overall morbidity in EP infants.

In interpreting these findings, clinicians should recognize that there is firm support to the American Academy of Pediatrics and other statements regarding the importance of human milk for preterm infants. Efforts should be developed to provide adequate donor milk to supplement mother's own milk so that these goals can be achieved.

Conclusions

The implications of these data are that efforts should be extended to provide as much human milk as possible to high-risk EP infants. The Surgeon General has advocated for expanding the use of donor milk that is pasteurized and safely provided to such infants.[1] Efforts to enhance the availability and affordability of pasteurized donor human milk should be increased. Our data suggest that the use of an exclusively human milk-based diet, using a nutritionally appropriate human milk-based fortifier, should be considered for all EP infants.

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Disclosure Statement

D.L.R. and M.J.L. are employees of Prolacta Bioscience. S.A.A. and R.J.S. declare no competing financial interests exist.

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Appendix

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