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Abstract

Objective

To investigate whether exposure to inhibitors of gastric acidity, such as H2 blockers or proton pump inhibitors, can independently increase the risk of infections in very low birth weight (VLBW) preterm infants in the neonatal intensive care unit.

Study design

This is a secondary analysis of prospectively collected data from a multicenter, randomized controlled trial of bovine lactoferrin (BLF) supplementation (with or without the probiotic *Lactobacillus rhamnosus* GG) vs placebo in prevention of late-onset sepsis (LOS) and necrotizing enterocolitis (NEC) in preterm infants. Inhibitors of gastric acidity were used at the recommended dosages/schedules based on the clinical judgment of attending physicians. The distribution of days of inhibitors of gastric acidity exposure between infants with and without LOS/NEC was assessed. The mutually adjusted effects of birth weight, gestational age, duration of inhibitors of gastric acidity treatment, and exposure to BLF were controlled through multivariable logistic regression.

Interaction between inhibitors of gastric acidity and BLF was tested; the effects of any day of inhibitors of gastric acidity exposure were then computed for BLF-treated vs -untreated infants.
Results
Two hundred thirty-five of 743 infants underwent treatment with inhibitors of gastric acidity, and 86 LOS episodes occurred. After multivariate analysis, exposure to inhibitors of gastric acidity remained significantly and independently associated with LOS (OR, 1.03; 95% CI, 1.008-1.067; $P=.01$); each day of inhibitors of gastric acidity exposure conferred an additional 3.7% odds of developing LOS. Risk was significant for Gram-negative ($P<.001$) and fungal ($P=.001$) pathogens, but not for Gram-positive pathogens ($P=.97$). On the test for interaction, 1 additional day of exposure to inhibitors of gastric acidity conferred an additional 7.7% risk for LOS ($P=.003$) in BLF-untreated infants, compared with 1.2% ($P=.58$) in BLF-treated infants.
Conclusion
Exposure to inhibitors of gastric acidity is significantly associated with the occurrence of LOS in preterm VLBW infants. Concomitant administration of BLF counteracts this selective disadvantage.

Keywords

lactoferrin
VLBW neonates
Candida
infection
colonization
H2 blockers

Abbreviations

BLF Bovine lactoferrin
FOS Fructo-oligosaccharides
H2B H2 blocker
LGG *Lactobacillus rhamnosus* GG
LOS Late-onset sepsis
NEC Necrotizing enterocolitis
NICU Neonatal intensive care unit
PPI Proton-pump inhibitor
RCT Randomized controlled trial
VLBW Very low birth weight

Introduction

Despite increased awareness and adoption of prophylactic measures, infections in preterm very low birth weight (VLBW) neonates in the neonatal intensive care unit (NICU) are frequent and are associated with substantial short-and long-term morbidity, increased health costs, and poor neurosensorial and neurodevelopmental outcomes in survivors.1, 2, 3 Accordingly, strategies that aim to prevent rather than treat infections have been developed, and include hygiene measures, use of central venous catheter infection prevention practices, pharmacologic and nutritional prophylaxis, and medical stewardship.4, 5

Despite the lack of class A evidence, inhibitors of gastric acid, such as H2 blockers (H2B; eg, ranitidine, cimetidine) and proton-pump inhibitors (PPIs; eg, omeprazole) have been widely used in recent decades in preterm infants to prevent upper gastrointestinal bleeding and to manage oral feeding intolerance or gastroesophageal reflux.[6]

Gastric acidity is a primitive, innate defensive system that decreases the density of ingested microorganisms passed pathogens from the stomach to the gut, hence decreasing the burden of microorganisms in the gut, which can disseminate into the bloodstream via an immature and leaky gut barrier.[7] Inhibitors of gastric acidity increase stomach pH, thereby decreasing the gastric acid barrier and potentially increasing the risk of systemic infection and necrotizing enterocolitis (NEC).8, 9, 10, 11, 12, 13

An association between exposure to inhibitors of gastric acidity and NICU infections has been suggested in a limited number of studies using a retrospective cohort or prospective observational design.9, 13, 14, 15, 16 The current level of evidence has some weaknesses and limitations. An association has been assessed using only a dichotomous approach (ie, exposure to inhibitors of gastric acidity: yes vs no). Moreover, the magnitude of association is uncertain, and the hypothesis of an association between inhibitors of gastric acidity and neonatal infections has not been tested using a prospective, randomized clinical trial design. Finally, it is not known whether any interaction exists between inhibitors of gastric acidity and concomitant prophylaxis with anti-infective medications.

In this study, we aimed to test the hypothesis that exposure to inhibitors of gastric acidity independently increases the risk of infections in preterm VLBW neonates. A second aim of this study was to explore the possible benefits of bovine lactoferrin (BLF) in preterm VLBW infants exposed to inhibitors of gastric acidity.

Methods

This is a secondary analysis of data obtained during a multicenter randomized controlled trial (RCT) (isrctn.org: ISRCTN53107700) performed in Italy and New Zealand from 2006 to 2010. The original study protocol, including structured criteria for inclusion/exclusion, enrollment, randomization, ethics, and institutional approvals, has been published previously.17, 18 In short, preterm VLBW neonates from 11 tertiary NICUs were enrolled before 72 hours of life and were randomly assigned to receive BLF alone (LF100; Dicofarm, Rome, Italy; 100 mg/day and 372 mg of fructo-oligosaccharides [FOS] per packet; group A1) or in combination with the probiotic *Lactobacillus rhamnosus* GG (LGG) (Dicoflor60; Dicofarm; 10^6 colony-forming units per day; group A2) or placebo (group B) from birth to day of life 30 (day of life 45 for those weighing <1000 g at birth). The drugs and placebo were administered orally, once daily. Neonates not feeding in the first 48 hours received the drug(s)/placebo by orogastric tube. The results from this RCT showed that BLF supplementation, either alone or in combination with LGG, reduces the risk of late-onset sepsis[18] and NEC[17] in VLBW infants compared with placebo.

In accordance with protocol, clinical and management data were collected prospectively for all enrolled infants until death or discharge. Systematic clinical surveillance for adverse events was performed through daily infant examination until 2 days after the end of treatment. Nutritional and feeding policies were stable during the study and consistent across centers, following common guidelines[19] and adherence to the study protocol.[18] Clinical surveillance for detection of sepsis was performed in all enrolled infants, with complete laboratory and microbiology evaluation in cases of suspected LOS.

In this secondary analysis, information regarding physicians' discretionary use, dosages, dosing schedules, and days of treatment of inhibitors of gastric acidity, as well as potential risk factors for infection, were reviewed from the study dataset, together with all data regarding fungal colonization and episodes of LOS.

The primary endpoint was the effect and magnitude of effect of inhibitors of gastric acidity exposure on the incidence of infections. We focused on the first episodes of LOS. Additional endpoints were to assess the effect of inhibitors of gastric acidity administration on fungal colonization and on NEC, the specific pathogens involved in the putative increased risk for sepsis attributable to inhibitors of gastric acidity, and whether oral supplementation of BLF (with or without the probiotic LGG) affects the occurrence of LOS and/or NEC in preterm VLBW neonates exposed to inhibitors of gastric acidity.

Inhibitors of gastric acidity were prescribed following common protocols and based on the clinical judgment of attending physicians, mainly for prevention of upper gastrointestinal bleeding (during medical treatment of patent ductus arteriosus) and management of oral feeding intolerance or gastroesophageal reflux in the enrolled infants. Dosages and schedules were the same for all participating centers, and followed current guidelines and recommendations. Ranitidine was administered at 0.5 mg/kg every 12 hours intravenously or 1-2 mg/kg every 8 hours orally. Cimetidine was administered at 2.5-5 mg/kg every 8-12 hours intravenously or orally. Omeprazole was dosed at 2-3 mg/kg/day. LOS was defined as an episode of infection occurring >72 hours after birth and before discharge, detected by clinical signs and symptoms, laboratory findings consistent with sepsis, and isolation of a causative organism from blood (drawn from peripheral sites), urine (collected by suprapubic puncture or bladder catheterization, with growth of >100 000 colony-forming units/mL [>10 000 for fungi]), or cerebrospinal or peritoneal fluid. Diagnostic criteria were based on the existing literature and guidelines from international consensus documents.[17, 20, 21, 22, 23]

Presumed sepsis (ie, clinical presentation consistent with sepsis without isolation of a microorganism) was not considered LOS, and thus was not analyzed in this study.

Systematic surveillance for detection of fungal colonization was performed through clinical and weekly surveillance cultures (at least 3 cultures per week from different peripheral sites) during the study period. Fungal colonization was defined as the detection of at least 1 surveillance culture positive for fungi during the NICU stay.

Standard laboratory methods were used to identify bacteria from cultures.[24, 25] For *Candida* species, specimens were incubated on chromogen culture plates (Albicans ID; bioMérieux, Marcy l'Etoile, France) to identify *Candida albicans* blue-staining colonies after 48 hours of incubation at 37 °C. Colonies were speciated biochemically (Vitec Yeast; bioMérieux).

NEC was defined as Bell stage ≥2, that is, clinical signs with the presence of pneumatosis intestinalis on abdominal radiographs.[26]

The criteria for hospital discharge were weight ≥1800 g, full oral feeding, and resolution of acute medical conditions.

Episodes of sepsis were treated with antibiotics/antifungal agents in accordance with the literature,[27] guidelines from international consensus documents, and recommendations of the Italian Neonatology Society's Fungal Infections Task Force.[22] Blinding was not broken to guide therapy.

Statistical Analyses

The association between exposure to inhibitors of gastric acidity (on any single day) and LOS was analyzed for all enrolled infants, and then separately for treatment groups (BLF/BLF+LGG vs placebo). All primary and secondary outcomes were analyzed on an intention-to-treat basis.

The variables analyzed were rates of LOS, invasive fungal infection, fungal colonization, NEC, and all-cause death before discharge. Groups A1 and A2 were compared separately to group B. Proportions and continuous variables were compared using the Fisher exact 2-tailed test and Student *t* test, respectively. The Wilcoxon Mann-Whitney test was used to compare the distribution of days of inhibitors of gastric acidity treatment between those with and without the outcome. Multivariable logistic regression was used to assess the mutually adjusted effects of variables significantly associated with LOS in univariate analysis: birth weight, gestational age, days of gastric acidity inhibitor treatment, exposure to BLF, and exposure to BLF+LGG. The interaction between inhibitors of gastric acidity and BLF was tested, and the effect of 1 extra day of treatment with inhibitors of gastric acidity was then computed for neonates treated with BLF and those not treated with BLF. Risk ratios (RRs) and 95% CIs were calculated to compare cumulative between-group incidences using Stata 13 (StataCorp, College Station, Texas). All tests were 2-tailed, and $P <$ .05 was considered to indicate statistical significance. All computations were performed using SPSS for Windows, version 16.0 (SPSS, Chicago, Illinois).

Results

A total of 743 VLBW neonates (247 in group A1, 238 in group A2, and 258 in group B) were enrolled and analyzed in the original trial.[18] Of these, 235 infants were treated with inhibitors of gastric acidity for at least 1 day and thus were eligible for this secondary analysis.

Demographics and clinical characteristics were similar in the 3 groups (**Table I**). The proportion of infants exposed to inhibitors of gastric acidity treatment, as well as the mean duration of the exposure to inhibitors of gastric acidity, were similar in all groups. The proportion of infants treated with inhibitors of gastric acidity was 31.1% in group A1 (77 of 247), 30.6% in group A2 (73 of 238), and 33.0% in group B (85 of 258) ($P = .99$ for both comparisons). Among the infants, PPIs were used in 4 of 77 in group A1, in 5 of 73 in group A2, and in 6 of 85 in groups B. Only 1 patient (in group B) received combined treatment with both classes of inhibitors of gastric acidity (H2B plus PPIs). The mean duration of inhibitors of gastric acidity treatment was 2.8 days in group A1, 3.2 days in group A2, and 3.3 days in group B ($P = .55$ and 0.99, respectively).

Table I. Demographic, clinical, and nutritional characteristics of the patients enrolled

Characteristics	Group A1	Group A2	Group B	*P* value
Number of patients (total, 743)	247	238	258	
Birth weight, g, mean ± SD, range	1158 ± 251, 605-1495	1129 ± 242, 550-1500	1118 ± 259, 437-1500	NS
Gestational age, wk, mean ± SD, range	29.7 ± 2.5, 23-36	29.6 ± 2.8, 23-35	29.4 ± 3.1, 23-39	NS

Characteristics	Group A1	Group A2	Group B	*P* value
Apgar score at 5 min, mean ± SD	7.4 ± 1.4	7.4 ± 1.7	7.5 ± 1.6	NS
Use of TPN, d, mean±SD	19.1 ± 20.1	17.7 ± 18.9	18.0 ± 17.0	NS
Intubation, d, mean±SD	5.3 ± 5.3	4.8 ± 6.0	4.9 ± 9.2	NS
Use of antibiotics, d, mean±SD	11.6 ± 8.9	11.7 ± 9.2	13.1 ± 10.0	NS
Use of postnatal early steroids, d, mean±SD	0.7 ± 0.8	0.5 ± 1.0	0.9 ± 1.1	NS
Central venous catheter, d, mean±SD	13.5 ± 10.5	13.4 ± 10.4	14.8 ± 11.8	NS
Mean stay in NICU, alive infants, d, mean±SD	53.5 ± 24.4	53.7 ± 22.3	51.7 ± 21.7	NS
Inhibitors of gastric acidity use, n	77	73	85	NS
Duration of inhibitors of gastric acidity, d, mean±SD	2.8 ± 6.1	3.2 ± 7.3	3.3 ± 7.5	NS
LOS per inhibitors of gastric acidity patients in each group, n	10	8	22	NS
Time of initiation of oral feeding, DOL, mean±SD	2.0 ± 2.9	2.0 ± 3.4	2.2 ± 3.4	NS
Time of achievement of full feeding, DOL, mean±SD	12.3 ± 4.3	13.2 ± 5.0	14.6 ± 4.9	NS
Volume of feedings advanced daily, mL/d, mean±SD	10.3 ± 4.7	11.2 ± 3.8	11.0 ± 3.5	NS
Infants fed with only formula milk, %	18.7	19.0	16.1	NS
Infants fed with only fresh human milk, %	27.7	27.1	29.0	NS
Infants fed with both formula and fresh human milk, %	53.4	51.8	49.0	NS
Daily fresh human milk intake, mL/kg, mean±SD	67.8 ± 42.9	64.1 ± 43.5	61.9 ± 40.0	NS
Total duration of fresh human milk feeding, d, mean±SD	21.7 ± 13.7	21.1 ± 14.1	21.8 ± 13.7	NS

DOL, day of life; *NS*, not significant; *TPN*, total parenteral nutrition.

Eighty-six first episodes of microbiologically confirmed LOS were diagnosed in the 743 VLBW infants enrolled. A total of 40 episodes of LOS occurred in the patients treated with inhibitors of gastric acidity (10 in group A1, 8 in group A2, and 22 in group B). The rates of LOS in these infants were 13% in group A1, 10.9% in A2, and 25.8% in B. After controlling for all variables significantly associated with infections, exposure to inhibitors of gastric acidity retained a significant and independent association with LOS (OR, 1.03; 95% CI, 1.008-1.067; *P*=.01) (**Table II**). Each additional day of therapy with inhibitors of gastric acidity conferred an additional 3.7% odds of developing develop infection.

Table II. Associations of outcomes of interest and exposure to inhibitors of gastric acidity

Outcomes	Mean exposure to inhibitors of gastric acidity in infants with the specific outcome, d	Mean exposure to inhibitors of gastric acidity in infants without the specific outcome, d	OR	95 CI	*P* value
Late-onset sepsis (microbiologically proven infection)	4.67	2.49	1.035	1.008-1.064	.014
Gram-positive infection	2.60	2.75	0.985	0.925-1.055	.69
Gram-negative infection	5.83	2.44	1.046	1.019-1.074	.01
Fungal infection	7.75	2.56	1.063	1.025-1.102	.01
Fungal colonization	5.48	2.27	1.045	1.021-1.070	.001
Death before discharge	1.90	2.77	0.947	0.872-1.029	.19
Threshold ROP	5.74	2.53	1.042	1.012-1.073	.006
NEC (Bell stage ≥2)	4.01	2.79	1.023	0.966-1.083	.044

ROP, retinopathy of prematurity.

Inhibitors of gastric acidity exposure was significantly associated with infection from Gram-negative ($P<.001$) and fungal ($P=.001$) pathogens, but not from Gram-positive organisms ($P=.97$). A significant association was observed for enteric fungal colonization as well ($P<.001$).

When analyzing the risk for infection in inhibitors of gastric acidity-treated infants according to BLF exposure (any type; test for interaction between BLF and inhibitors of gastric acidity), it was noted that each day of exposure to inhibitors of gastric acidity conferred an additional 7.7% risk for infection in infants not exposed to BLF ($P=.003$), compared with 1.2% ($P=.58$) in those receiving BLF (with or without LGG) (**Table III**).

Table III. Test for interaction between BLF and inhibitors of gastric acidity

Outcome	Test for interaction	OR	*P* value	95% CI
	Interaction (BLF and inhibitors of gastric acidity)	0.939	.056	0.881-1.002
Infection	Effect of 1 more d of inhibitors of gastric acidity in non–BLF-treated infants	1.077	.003	1.026-1.131
	Effect of 1 more d of inhibitors of gastric acidity in BLF-treated infants	1.012	.585	0.970-1.055
	Interaction (BLF and inhibitors of gastric acidity)	0.937	.019	0.888-0.989
Fungal colonization	Effect of 1 more d of inhibitors of gastric acidity in non–BLF-treated infants	1.098	.000	1.047-1.151
	Effect of 1 more d of inhibitors of gastric acidity in BLF-treated infants	1.029	.035	1.002-1.057
	Interaction (BLF and inhibitors of gastric acidity)	0.816	.043	0.670-0.994
NEC (≥2nd stage)	Effect of 1 more d of inhibitors of gastric acidity in non–BLF-treated infants	1.214	.021	1.030-1.431
	Effect of 1 more d of inhibitors of gastric acidity in BLF-treated infants	0.991	.866	0.886-1.107

Note: This test was performed on all patients receiving BLF, regardless of concomitant administration of the probiotic LGG, because an additive effect of this last compound on the incidence of LOS was not demonstrated.

A similar pattern was found for the relationships with fungal colonization and NEC (**Table III**). Importantly, 1 additional day of exposure to inhibitors of gastric acidity conferred an additional 11.4% risk of NEC among infants not treated with BLF, whereas this risk decreased to 0 for BLF-treated infants.

No adverse effects or treatment intolerance occurred related to BLF administration.

Discussion

This study provides prospectively generated evidence that VLBW infants exposed to inhibitors of gastric acidity have significantly increased odds of developing infections, and that the risk increases with each day of exposure to inhibitors of gastric acidity. Our data also reveal that BLF or BLF+LGG exposure counteracts the risk for infections related to inhibitors of gastric acidity. In this study, the main pathogens causing infections in infants exposed to inhibitors of gastric acidity were Gram-negative bacilli and *Candida* spp. In addition, exposure to inhibitors of gastric acidity increased the risk of *Candida* spp colonization in the gut. The main reservoir of these 2 groups of microorganisms is the gut, unlike for many Gram-positive organisms. Our findings of increased LOS due to these 2 classes of pathogens are consistent with advantage provided by an impaired acid barrier. This is also in line with reports suggesting that *Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa* are the main pathogens isolated in infants exposed to inhibitors of gastric acidity.[15] *Candida* colonization is the most potent risk factor for *Candida* systemic infection and the

enteric reservoir is the main source for systemic dissemination in immunologically compromised patients such as preterm infants. A relevant body of evidence supports the use of fluconazole to prevent *Candida* colonization and infections in preterm infants.28, 29, 30, 31 In our previously reported results of a multicenter RCT of fluconazole prophylaxis in preterm infants, fluconazole was 70% effective in preventing *Candida* colonization.[31] The present study shows that each day of exposure to inhibitors of gastric acidity confers a 4.5% additional risk of acquiring *Candida* colonization in preterm infants, and that concomitant administration of BLF (alone or with LGG) significantly reduces this risk. Strategies aimed at preventing *Candida* colonization and infection should consider withdrawal of inhibitors of gastric acidity to avoid *Candida* colonization, and studies should be conducted to verify whether fluconazole-based prophylactic strategies are hampered by the concomitant use of inhibitors of gastric acidity.

The progressive time-related increase in the risk of infection, whether bacterial or fungal, determined by exposure to inhibitors of gastric acidity suggests that it may be worth investigating whether the duration and/or dosage of inhibitors of gastric acidity treatment in VLBW neonates should be reduced progressively after day 1. On the other hand, the similar decrease in risk of infection determined by BLF irrespective of duration of inhibitors of gastric acidity treatment suggests that such a strategy might not be necessary, providing that BLF treatment accompanies inhibitors of gastric acidity treatment. All the same, inhibitors of gastric acidity treatment may reduce an otherwise greater beneficial effect of BLF treatment on reduction of infection. A recent Cochrane Review reports that BLF reduces the frequency of infections in VLBW neonates in the NICU by 51%.[32] The fact that BLF almost entirely counteracts the disadvantage of inhibitors of gastric acidity but does not further decrease the risk of infection to the level of neonates treated with BLF who are not exposed to inhibitors of gastric acidity suggest that the beneficial effect of BLF is somewhat mitigated by the detrimental effect of inhibitors of gastric acidity.

Our data confirm, strengthen, and expand on previous reports describing an association between inhibitors of gastric acidity use and infections9, 13, 14, 15, 16 through its prospective and international multicenter design. In addition, the overall magnitude of the association and its specificity by pathogens was examined with variables related to local clinical practice were bypassed. Terrin et al[15] assessed the risk of infection comparing infants exposed to ranitidine vs unexposed, but considered as exposed only those infants receiving at least 7 days of treatment. In the present era, most infants in the NICU are treated with inhibitors of gastric acidity only in specific circumstances, and only for a few days.

Our study also shows that exposure to inhibitors of gastric acidity is independently associated with the occurrence of NEC, and that concomitant treatment with BLF mitigates this risk significantly. The results of our study expand some previously published evidence.15, 18 Because in our study, exposure to fresh human milk was comparable in the 3 groups, we excluded that the increased risk of developing NEC or LOS in infants exposed to inhibitors of gastric acidity was due to differences in exposure to nutrients that are known to affect the occurrence of infections and NEC.

BLF reduces the rate of progression from enteric colonization with *Candida* spp to systemic infection.[33] At high concentrations, BLF enhances maturation of the gut barrier by increasing the number of gut cells, promoting the closure of enteric gap junctions, thus reducing intestinal permeability and dissemination of gut organisms into the bloodstream.34, 35, 36, 37 This explanation is in line with the similar beneficial effect of BLF on bacterial[33] and fungal causes of infection.[38] In addition, BLF is also bifidogenic,[39] thus contributing to the establishment of an advantageous microflora in the gut. We speculate that BLF's protective role in infants exposed to inhibitors of gastric acidity is a dual one. Furthermore, BLF has well-known antioxidative actions

through reduction of free radicals of oxygen in the gut,40, 41 which might play a key role in this context.

In the present study, the BLF product contained some amounts of FOS. Because FOS have actions similar to prebiotics or human oligosaccharides, we cannot exclude that the FOS contained in the BLF product might have affected the microbiome and ultimately contributed to the reported modifications in the pathogens' gut ecology in addition to the effect of lactoferrin.

A limitation of this study is that the results are derived from a post hoc analysis of data from an RCT with a different primary outcome assessed. It is difficult to plan an RCT of inhibitors of gastric acidity use. Further investigation of the pathogenesis of infections in infants exposed to inhibitors of gastric acidity is warranted. Possibly, increased information on the mechanisms of gut colonization and on the development of enteric inflammation in such infants will provide a better understanding of the role of preventive strategies and the extent of the protective effect of BLF. Meanwhile, because even 1 day of exposure to inhibitors of gastric acidity confers additional, quantifiable risk of LOS, it seems prudent that inhibitors of gastric acidity be administered when only strictly necessary, over the shortest possible course, and with consideration of concomitant administration of BLF in an attempt to counteract adverse effects associated with the use of inhibitors of gastric acidity.

Appendix

Additional members of the Italian Task Force for the Study and Prevention of Neonatal Fungal Infections, affiliated with the Italian Society of Neonatology, includes the following institutions and individuals:

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