

The Protective Role of Gastric Acidity in Neonatal Bacterial Translocation

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● The acid environment of the stomach serves as an important defense against intestinal colonization by potentially pathogenic bacteria. The purpose of this study was to examine the effect of increased gastric pH on bacterial translocation in a neonatal rabbit model. Fifty-nine rabbit pups were delivered by cesarean section and randomly divided into normal acid (NA) and reduced acid (RA) groups. All were gavage fed and challenged with *Enterobacter cloacae*, 1×10^6 CFU/mL. The RA group received ranitidine, 20 mg/kg/d with all feeds. Gastric pH was measured by pH probe before and 4 hours after bacterial challenge. Mesenteric lymph node (MLN), spleen, liver, midjejunum, and cecum were harvested for culture at 72 hours. Gastric pH in the RA group was significantly increased before and 4 hours after the bacterial challenge. The incidence of bacterial translocation to the MLN, spleen, and liver was significantly higher in the RA group. Log cecal and jejunal colony counts were significantly increased in the RA animals. The authors conclude that the gastric acidity is protective against intestinal colonization and translocation of potentially pathogenic bacteria in this neonatal rabbit model.

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INDEX WORDS: Bacterial translocation, gastric acidity, ranitidine.

MANY CRITICALLY ILL newborns, especially preterm infants, are at risk for bacterial sepsis. One of the increasingly important routes of bacterial invasion requires colonization, which allows bacterial translocation through the gastrointestinal mucosal barrier. The acid environment of the stomach serves as an important defense against intestinal colonization by potentially pathogenic bacteria. The purpose of this study was to examine the effect of inhibiting gastric acid on bacterial translocation in a neonatal rabbit model.

MATERIALS AND METHODS

Sixty-two New Zealand white rabbit pups weighing greater than 40 g were delivered by sterile cesarean section one day preterm. The pups were placed in sterile nesting boxes and randomly placed into one of two groups: a normal gastric acid (NA) control group or a reduced

gastric acid (RA) experimental group. Gastric acid was reduced in the experimental group by the addition of ranitidine hydrochloride to all feedings.

All animals were kept in an environmentally controlled room. Beginning 6 hours after delivery, all animals were gavage fed artificial formula (KMR, Pet-Ag, Inc, Elgin, IL) twice daily using a 3.5F umbilical artery catheter. Feeds were started at 80 kcal/kg/d and increased by 10 kcal/kg/d for the duration of the feeding schedule. The RA group had ranitidine syrup (15 mg/mL; Glaxo Pharmaceuticals, Research Triangle Park, NC) added to all feeds to receive 20 mg/kg/d. All animals were challenged with 1×10^6 CFU/mL of *Enterobacter cloacae* mixed with the third feeding. The source of the *E. cloacae* was from a septic neonate at Arkansas Children's Hospital. The bacteria is kept in stock on glass beads in a -70°C freezer. Quality assurance of the bacteria for each experiment includes purity plates, analytical profile index (API) identification (bioMérieux Vitek Inc, Hazelwood, MO), and testing for type I fimbria by guinea pig agglutination. Bacterial suspensions are prepared to a concentration of 3×10^9 CFU/mL by comparison with a #10 McFarland standard. The suspension is then diluted serially to achieve bacterial concentration of 1×10^6 CFU/mL. Quantitative culture is performed to determine the actual concentration of the challenge. Previous studies in our laboratory have shown this *E. cloacae* to translocate at a reproducible rate in this neonatal rabbit model.

Intraluminal gastric pH level was measured by placing a neonatal pH probe (Synetics Medical, Irving, TX). Gastric pH level was measured before the first feeding, and before and 4 hours after the bacterial challenge in all animals.

All animals were killed 40 hours after the bacterial challenge by intracardiac administration of pentobarbital. The animals' abdomens were opened using sterile technique. Peritoneal swabs were taken and culture tests performed for gram-negative and gram-positive bacteria. All animals with positive peritoneal swab findings were considered contaminated and were excluded from analysis. The mesenteric lymph node (MLN), liver, spleen, and cecum were harvested and placed in previously weighed sterile petri dishes. The experiment was performed on two separate occasions because of the number of animals that could be cared for at one time. The second time the experiment was performed the midjejunum was also harvested for culture to assess the quantity of bacteria in the proximal bowel. The organ weights were recorded, and sterile trypticase soy broth (TSB, 9 mL/g tissue) was placed in the dish with the tissue. Tissue was homogenized in the broth, and a 1-mL aliquot was culture-tested for aerobic gram-positive and gram-negative bacteria. One hundred microliters of the homogenate was placed in a TSB tube and incubated. The broths were replated if they appeared positive or after 72 hours. This was done to increase the theoretical limit of detection of microorganisms to one per organ. API identification was performed on randomly selected positive plates.

Table 1. Incidence of Bacterial Translocation of *Enterobacter cloacae*

Organ	NA Group (n = 20)	RA Group (n = 39)
MLN	3 (15%)	22 (56%)*
Liver	4 (20%)	21 (54%)*
Spleen	3 (15%)	22 (56%)*

* $P < .02$.

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Table 2. Bacterial Colonization and Quantitative Log Counts

Intestine	Bacteria	NA Group (n = 20, cecum) (n = 8, jejunum)	Mean Log Count	RA Group (n = 39, cecum) (n = 15, jejunum)
Cecum	<i>E cloacae</i>	20 (100%)	9.04 ± 0.40	39 (100%)
	<i>S epidermidis</i>	0		15 (38%)*
	<i>Staphylococcus</i> (coagulase-positive)	0		1 (3%)
Jejunum	<i>E cloacae</i>	6 (75%)	6.40 ± 0.47	15 (100%)
	<i>S epidermidis</i>	0		2 (13%)

* $P < .01$.

Incidence of translocation to the MLN and organs was compared between groups using the Fisher's Exact test. Quantity of bacteria in the MLN, liver, spleen, cecum, and jejunum was reported as means ± standard deviation of the log count and analyzed using the Student's *t* test.

RESULTS

There were three positive peritoneal swab test results, all in the RA group. These animals were excluded from the following results. Before the first feeding, the gastric pH was similar between the RA and NA groups (3.6 ± 0.6 v 3.6 ± 0.4). Gastric pH in the RA group was significantly increased before (4.7 ± 0.6 v 3.1 ± 0.7) and 4 hours after the bacterial challenge (5.0 ± 0.5 v 3.4 ± 0.6), $P < .01$. The incidence of bacterial translocation to the MLN, spleen, and liver was significantly higher in the RA group (Table 1). The log quantity of bacteria that translocated to the MLN, spleen, and liver in the RA and NA groups were not significantly different. Log cecal (9.63 ± 0.58 v 9.04 ± 0.40) and jejunal (8.63 ± 0.66 v 6.40 ± 0.47) colony counts of *E cloacae* were significantly increased in the RA animals. The incidence of colonization of the cecum with *Staphylococcus epidermidis* was also significantly higher in the RA group (Table 2).

DISCUSSION

The acid environment of the stomach serves as an important defense against intestinal colonization by potentially pathogenic bacteria. Gastric acid secretion is present before birth in the fetal rabbit.¹ A previous experiment in our laboratory suggested that *E cloacae* does not tolerate acidic pH (4.0) but proliferates in more neutral pH (6.5) over a 4-hour period. These findings are

supported by many clinical studies that concluded that increased gastric pH increases gastric colonization of bacteria.²⁻⁹ Jejunal bacterial overgrowth is also associated with decreased gastric acidity.^{5,10}

Ranitidine is a histamine-2 receptor antagonist used to prevent gastric bleeding in critically ill patients by inhibiting gastric acidity.¹¹⁻¹³ Recent experimental and clinical study results have suggested that inhibiting gastric acidity increases the risk for developing nosocomial pneumonia.^{4,7,14-16}

Our study results demonstrate that ranitidine increased gastric pH in neonatal rabbits. This decreased acidity allowed bacterial overgrowth in the cecum and jejunum and subsequently increased bacterial translocation to the MLNs, spleen, and liver. Coagulase-negative staphylococci, and particularly *S epidermidis*, have become the major nosocomial pathogens in neonates.¹⁷ The RA group had colonization of the cecum and jejunum with *S epidermidis* with occasional translocation to the MLN, liver, and spleen in a few animals. The NA group had no colonization or translocation with *S epidermidis*. This suggests that gastric acidity is protective against colonization and translocation by both gram-positive and gram-negative organisms.

Histamine-2 receptor antagonists are used extensively in the neonatal intensive care units on patients who already have a number of risk factors for the development of sepsis.

Our data demonstrate that gastric acidity is protective against intestinal colonization, bacterial overgrowth, and translocation in our neonatal rabbit model. This suggests that inhibiting gastric acidity may predispose neonates to nosocomial pneumonias and systemic sepsis.

REFERENCES

1. Yee LF, Andrews KJ, Calauastro EQ, et al: Mechanisms of gastric acid secretion in the fetal rabbit. *Surgery* 118:199-205, 1995
2. Noguchi J, Konishi M, Tatara T, et al: The effects of H₂ blockers on gastric flora and sputum culture. *Japn J Anes* 43:1243-1247, 1994
3. Bonten MJ, Guillard CA, Vantel FH, et al: Continuous enteral feeding counteracts preventive measures for gastric colonization in intensive care patients. *Crit Care Med* 22:939-44, 1994
4. Prod'ham G, Leuenberger P, Koefer J, et al: Nosocomial pneumonia in mechanically ventilated patients receiving antacid, ranitidine, or sucralfate as prophylaxis for stress ulcer. A randomized controlled trial. *Ann Intern Med* 120:653-662, 1994
5. Shindo K, Machida M, Miyakawa K, et al: A syndrome of cirrhosis, achlorhydria, small intestinal bacterial overgrowth, and fat malabsorption. *Am J Gastroenterol* 88:2084-2091, 1993
6. Husebye E, Skar V, Hoverstad T, et al: Abnormal intestinal motor patterns explain enteric colonization with gram negative bacilli in late radiation enteropathy. *Gastroenterol* 109:1078-1089, 1995
7. Tryba M, Cook DJ: Gastric alkalinization, pneumonia, and

systemic infections: The controversy. *Scand J Gastroenterol* 210:53-59, 1995

8. Boten MJ, Gaillard CA, Vandergeest S, et al: The role of intragastric acidity and stress ulcer prophylaxis on colonization and infection in mechanically ventilated ICU patients. A stratified, randomized, double-blind study of sucralfate versus antacids. *Am J Respir Crit Care Med* 152:1825-1834, 1995

9. Sirvent JM, Verdager R, Ferrer MJ, et al: Mechanical ventilation-associated pneumonia and the prevention of stress ulcer. A randomized clinical trial of antacids and ranitidine versus sucralfate. *Med Clin* 102:407-411, 1994

10. Shindo K, Fukumura M: Effect of H₂-receptor antagonists on bile acid metabolism. *J Invest Med* 43:170-177, 1995

11. Osteyee JL, Banner W Jr: Effects of two dosing regimens of intravenous ranitidine on gastric pH in critically ill children. *Am J Crit Care* 3:267-272, 1994

12. Fontana M, Tornaghi R, Petrillo M, et al: Ranitidine treatment in

newborn infants: Effects on gastric acidity and serum prolactin levels. *J Ped Gastroenterol Nutr* 16:406-411, 1993

13. Gedeit RG, Weigle CG, Havens PL, et al: Control and variability of gastric pH in critically ill children. *Crit Care Med* 21:1850-1855, 1993

14. Apte NM, Karnad DR, Medheka, et al: Gastric colonization and pneumonia in intubated critically ill patients receiving stress ulcer prophylaxis: A randomized, controlled trial. *Crit Care Med* 20:590-593, 1992

15. Cook DJ, Reeve BK, Guyatt GH, et al: Stress ulcer prophylaxis in critically ill patients. Resolving discordant meta-analysis. *JAMA* 275:214, 1996

16. Bezarro ER: Changing perspectives of H₂ antagonists for stress ulcer prophylaxis. *Crit Care Nurs Clin North Am* 5:325-331, 1993

17. Stoll BJ, Gordon T, Korones SB, et al: Late onset sepsis in very low birth weight neonates: A report from the National Institute of Child Health and Human Development Neonatal Research Network. *J Pediatr* 129:63-71, 1996

Discussion

B. Harden (Birmingham, AL): This elegant study demonstrates the effects of reduced gastric acid on bacterial translocation in this neonatal rabbit model.

Bacterial translocation is being increasingly implicated in the multiorgan failure syndrome seen in patients with multiple injuries, major burns, and sepsis. Intestinal translocation may also occur in a controlled fashion presenting the gut-associated lymphoid tissue with an antigenic challenge, which promotes immunocompetence.

This study also demonstrates the importance of gastric acid in controlling upper gastrointestinal microbial colonization with the oral microflora.

Did you perform blood cultures or examine lung or other tissues to determine if this bacterial translocation was in any way injurious to the host?

In view of the size of the bacterial challenge, would you speculate on why only half of the animals in the ranitidine group had positive mesenteric node, liver, and spleen cultures?

On the basis of this study, what would be your recommendations regarding the use of H-2 blockers or

antacids in our neonatal population? Would you consider the use of carafate as a possible alternative method of treating these patients?

J.E. Dinsmore (response): We did not take blood cultures in these animals. We sampled the lung tissue in some animals and found that the reduced acid group had increased positive cultures in the lung compared with the control group.

Only half the animals in the reduced acid group had colonization or translocation to the organs. More bacteria may translocate, but not remain viable because of the mucosal defense mechanisms. The bacteria that we culture are the bacteria that have survived.

Regarding carafate or antacids, there are many studies that have been performed in critically ill adults comparing carafate antacids, and H-2 blockers. The carafate patients had less nosocomial pneumonia and lower mortality.

The next step of this study would be to do a similar randomized control study in neonatal intensive care units.