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 divided into normal acid (NA) and reduced acid (RA) groups. Gastric acid was reduced in the RA experimental group by the addition of ranitidine hydrochloride to all feedings. 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 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 suspension is 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.

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| 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.
PROTECTIVE ROLE OF GASTRIC ACIDITY

Table 2. Bacterial Colonization and Quantitative Log Counts

<table>
<thead>
<tr>
<th>Intestine</th>
<th>Bacteria</th>
<th>NA Group (n = 20, cecum)</th>
<th>Mean Log Count</th>
<th>RA Group (n = 30, cecum)</th>
<th>Mean Log Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cecum</td>
<td><em>E. cloacae</em></td>
<td>20 (100%)</td>
<td>9.04 ± 0.40</td>
<td>29 (100%)</td>
<td>15 (38%)</td>
</tr>
<tr>
<td></td>
<td><em>S. epidermidis</em></td>
<td>0</td>
<td></td>
<td></td>
<td>1 (3%)</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus</em></td>
<td>0</td>
<td></td>
<td></td>
<td>2 (13%)</td>
</tr>
<tr>
<td>Jejunum</td>
<td><em>E. cloacae</em></td>
<td>6 (75%)</td>
<td>6.40 ± 0.47</td>
<td>15 (100%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>S. epidermidis</em></td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*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. 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. Jejunal bacterial overgrowth is also associated with decreased gastric acidity.

Ranitidine is a histamine-2 receptor antagonist used to prevent gastric bleeding in critically ill patients by inhibiting gastric acidity. Recent experimental and clinical study results have suggested that inhibiting gastric acidity increases the risk for developing nosocomial pneumonia.

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. The RA group had colonization of the cecum and jejunum with *S. epidermidis* with occasional translocation to the MLNs, spleen, and liver. The RA 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

7. Tylsen H, Cook DJ: Gastric alkalization, pneumonia, and


**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 microbiota.

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.