Clindamycin-Associated Enterocolitis in Guinea Pigs: Evidence for a Bacterial Toxin

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Experimental enterocolitis was induced in guinea pigs by intraperitoneal injection of clindamycin. Specimens of feces were collected daily in phosphate-buffered saline (pH 7.0) and pooled every second day. The pooled samples were centrifuged to remove solids, and the supernatant was sterilized by membrane filtration. The sterile fecal supernatants were then dialyzed for 48 h against two 15-liter changes of phosphate-buffered saline and subsequently tested for toxicity in cultured monolayers of mouse adrenal cells. A filterable toxin(s) was found in the fecal supernatants on days 2, 4, and 6 postchallenge and not in pretreatment samples. The toxin(s) caused enterocolitis when administered orogastrically to healthy animals and altered the morphology of cultured mouse adrenal cells. The alteration of adrenal cell morphology was neutralized by specific antitoxin to Clostridium histolyticum.

The administration of clindamycin or lincomycin to hamsters or guinea pigs results in a high incidence of lethal enterocolitis (2, 3, 10). Although the pathogenesis has remained unclear, recent studies have implicated the role of a bacterial toxin in antibiotic-associated enterocolitis (2, 5, 8). Bartlett et al. (2) have shown that clindamycin-associated colitis in hamsters is due to a clindamycin-resistant, toxin-producing strain of clostridium similar to Clostridium difficile. Larson and Price (7) and Rifkin et al. (8) were able to neutralize a toxin found in the feces of nine patients with enterocolitis by antitoxin to C. sordelli. The toxin was not neutralized by antitoxin to C. welchii, C. oedematiens, or C. septicum.

The results of this study demonstrate the presence of a cytotoxin in the feces of guinea pigs with clindamycin-associated enterocolitis. The toxin induced enterocolitis when administered orogastrically to healthy animals, altered the morphology of cultured mouse adrenal cells, and was neutralized by specific antitoxin to C. histolyticum.

MATERIALS AND METHODS

Drug challenge. An experimental group of six albino guinea pigs (200 g) were challenged daily intraperitoneally with 15 mg of clindamycin in 0.25 ml of sterile phosphate-buffered saline (PBS) at pH 7.0. A control group received an equal volume of diluent without antibiotic. Each group of animals was housed in a separate stainless-steel cage.

Collection of feces. Feces were collected daily in the early morning by addition of 100 ml of PBS to the cage collecting pans. The fecal samples were dispersed in the buffer with a glass rod and stored at 4°C. The samples were pooled every 2 days and centrifuged for 18 h at 12,000 x g (4°C). The fecal supernatant (FS) was then filter sterilized (0.20 μm, Millipore Corp., Bedford, Mass.) and dialyzed for 48 h against PBS (pH 7.0). Sterile FS was tested for toxic activity in cultured monolayers of mouse adrenal cells and for induction of enterocolitis in healthy guinea pigs.

Cultured cell procedures. Tissue culture procedures were conducted as described elsewhere (4). Briefly, mouse adrenal cell monolayers were cultured in minimal essential medium (GIBCO, Grand Island, N.Y.) supplemented with 10% fetal calf serum at 37°C in a humidified atmosphere of 5% CO₂ in air. Cultured monolayers (85% confluency) in 25-cm² plastic flasks were challenged with twofold serial dilutions of sterile FS in tissue culture medium. Morphological responses were observed after 24 h of incubation, and toxin titers were defined as the highest dilution that caused in excess of 90% cell rounding (4). Toxin neutralization tests were performed by testing the ability of specific antitoxin to neutralize the toxic effect on cultured mouse adrenal cells. Antitoxin to C. sordelli, C. perfringens (type A), C. septicum, C. histolyticum, and C. oedematiens (American Cyanamid Co., Lederle Laboratories Div., Pearl River, N.Y.) was tested in the cell culture assay by the method of Bartlett et al. (1).

Orogastric challenge. The orogastric administration of sterile fecal extracts containing toxic activity to otherwise healthy guinea pigs was performed by the method of Rifkin et al. (9). Briefly, sterile FS (3.0 ml) was administered orogastrically to etherized guinea pigs (n = 4) via 9-cm catheters. A control group received an equal volume of either PBS (pH 7.0) or sterile FS from pretreatment samples.
RESULTS AND DISCUSSION

All animals challenged with clindamycin appeared acutely ill and moribund on day 2 (Table 1). On subsequent days they showed weight loss, reduced water intake, and lethargy. Experimental animals expired during the investigation on days 6 and 8 postchallenge. Postmortem examinations revealed an enlarged small bowel and massive cecal dilatation; the latter showed a number of hemorrhages. Changes in the quantity of intraperitoneal fluid were not observed. Katz et al. (5) and Bartlett et al. (2) have shown similar findings in rabbits and hamsters.

Adrenal cell assay of FS showed a relatively high level of toxic activity on days 2 and 4, with a decrease on day 6. No toxic activity could be detected in FS on day 8 or in pretreatment samples (Table 1). Although the decrease in toxic activity on days 6 and 8 cannot be explained and occurred during a time period when lethality resulted, the absorption of toxic activity to intestinal mucosa or systemic uptake is possible. Infectious enterotoxemia of mammals and enteritis of fowl have been described and shown to result from the absorption of a clostridial toxin from the intestine (11). The toxic activity observed on days 2, 4, and 6 was found to be heat labile (56°C, 60 min) and retained by an ultrafiltration membrane having a nominal molecular weight cutoff of 10,000 (Amicon Corp., Lexington, Mass.).

Further, the orogastric administration of toxic FS (days 2 and 4) to healthy guinea pigs resulted in the appearance of enterocolitis within 2 days. At autopsy massive cecal dilatation and enlargement of the small bowel were prominent. All control animals remained well and showed no abnormal pathology at autopsy. Similar results on heat lability, ultrafiltration, and serial animal passage have been shown in the hamster model (1, 9).

This investigation extends the findings of Larson et al. (6, 7) and Rifkin et al. (8, 9) that a heat-labile toxin(s) is suspect in the etiology of enterocolitis. These results suggest that the guinea pig derived toxin is from C. histolyticum. This toxin was cytotoxic for mouse adrenal cells and could be specifically neutralized. Antitoxin to C. histolyticum, but not to other clostridial species, neutralized the toxicity of FS samples collected on days 2, 4, and 6 in adrenal cell assays (Table 2). However, it should be noted that the major toxin (alpha) produced by C. histolyticum is neutralized by antisera to C. septicum. Additionally, the neutralization of C. difficile toxin, the etiological agent of hamster enterocolitis, by C. sordellii antisera is documented (9). Apparently, the antigenic cross-reactivity among clostridial toxins is not uncommon.

The cytotoxic effect on cultured adrenal cell monolayers, heat lability, and findings at autopsy resemble the properties of C. histolyticum toxin(s) which cause edema and hemorrhage. The hemorrhagic liquefaction of soft tissue and proteolytic capabilities of C. histolyticum are well documented (11). Cultural studies related to the isolation of C. histolyticum from symptomatic and normal guinea pigs are in progress. These studies are being conducted to isolate the causative agent and assess its role in human disease.

Although these experiments are inconsistent

### Table 1. Comparison of tissue culture toxicity from guinea pig fecal extracts

<table>
<thead>
<tr>
<th>Day</th>
<th>Clinical state</th>
<th>Tissue culture toxicity</th>
<th>Relative toxicity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Well</td>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Acutely ill</td>
<td>Positive</td>
<td>2,048</td>
</tr>
<tr>
<td>4</td>
<td>Acutely ill</td>
<td>Positive</td>
<td>1,024</td>
</tr>
<tr>
<td>6</td>
<td>Acutely ill,</td>
<td>Positive</td>
<td>32</td>
</tr>
<tr>
<td>8</td>
<td>Death</td>
<td>Negative</td>
<td>0</td>
</tr>
</tbody>
</table>

*Data represented is from six guinea pigs; feces were pooled every second day. Zero (0) represents feces collected before drug challenge.

*Represents the reciprocal of the highest dilution of sterile fecal supernatant that produced in excess of 90% cell rounding in 24 h; toxicity in FS was completely neutralized by antitoxin to C. histolyticum. See text for details of the experiment.

### Table 2. Neutralization of tissue culture toxicity by clostridial antitoxin

| Cytopathic effect produced by fecal suspensions incubated with antitoxin to: |
|-------------------------------|-------------------|------------------|--------------------|
| Day                          | C. sordellii     | C. perfringens*  | C. septicum       | C. histolyticum    | C. oedematus       |
| 0                            | -                 | -                | -                  | -                  | -                 |
| 2                            | +                 | +                | +                  | +                  | +                 |
| 4                            | +                 | +                | +                  | -                  | +                 |
| 6                            | +                 | +                | +                  | +                  | -                 |
| 8                            | -                 | -                | -                  | -                  | -                 |

*Type A.

b Positive (+) indicates in excess of 90% cell rounding in adrenal cell assay; negative (−) indicates the absence of morphological changes. See text for details of the experiment.
with other recent studies that implicate *C. difficile* as the etiological agent (1, 2, 5–9), they confirm and extend other observations that pseudomembranous colitis is induced by a clostridial toxin. Whether the animal species determines which clostridia will overgrow in the intestine as a result of exposure to clindamycin is not known. If the animal species is important, then the microbial toxin specifically responsible for the human disease can only be established definitively by human studies. Further investigations will be required to determine the role of various clostridia in the etiology of the human disease and to determine whether the induction of disease by different antibiotics is related to a single clostridial species.

These results on experimental clindamycin-associated enterocolitis and the observations of others (1–10) lend support to the hypothesis that this disease is mediated by yet another bacterial toxin.

**LITERATURE CITED**