Etiology of Tetracycline-Associated Pseudomembranous Colitis in Hamsters

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Tetracyclines were implicated in the 1950s in induction of protracted diarrhea and pseudomembranous colitis (PMC) in humans (1, 3, 8, 12, 17). PMC was thought to occur because the normal gastrointestinal flora was altered, with concomitant overgrowth of enterotoxigenic Staphylococcus aureus resistant to tetracycline. Recently, whether S. aureus is an etiology of PMC has been debated partly because cases have occurred despite negative stool cultures for S. aureus (3). In addition, some investigators have treated antibiotic-induced diarrhea successfully with oral tetracycline (9, 16).

Research into the cause of PMC has been stimulated by the appearance of many cases related to clindamycin and lincomycin. As a result, it now appears that clindamycin-induced PMC is caused by toxigenic Clostridium difficile resistant to clindamycin. The role of this organism in the causation of PMC was documented by study of hamsters challenged with antibiotics. It is apparent this animal provides a good model of antibiotic-induced PMC in humans. We used this model to reexamine the etiology of PMC induced by tetracycline and to explore the protective role of tetracycline against PMC induced by other antibiotics.

MATERIALS AND METHODS

Male golden Syrian hamsters (60 to 100 g) obtained from Charles River Breeding Laboratories (Newfield, N.J.) were housed 8 to 10 per cage in an air-conditioned room (20°C), fed Teklad 1148 Rat-Mouse Diet, and provided tap water.

Tetracycline hydrochloride (Pfizer, New York, N.Y.) was diluted to desired concentrations with sterile 0.9% sodium chloride (normal saline) and administered oro-gastrically under light ether anesthesia by 16-gauge polyethylene catheters. A single administration of tetracycline in amounts of 1, 10, or 100 mg/kg was given. Tetracycline also was given similarly in 10- or 100-mg/kg daily doses for 3, 7, or 14 days. Control animals received normal saline in equal volumes. Hamsters challenged with a single oro-gastric administration of 10 mg of clindamycin phosphate (Upjohn, Kalamazoo, Mich.) per kg were promptly given oro-gastric tetracycline, 10 or 100 mg/kg, or normal saline daily for 3, 7, or 14 days.

All animals were inspected and weighed daily, and postmortem examinations were performed on all dead animals. Cecal contents were obtained for bacteriological study (15). Hamster cecal contents were diluted with an equal volume of sterile saline and centrifuged at 500 × g for 20 min. The crude supernatant fluids were then centrifuged at 10,000 × g for 30 min at 4°C, and the supernatant was passed through 0.45-μm membranes (Millipore Corp.) to obtain sterile filtrates of cecal contents. These filtrates were then tested for the presence of clostridial toxin in hamsters and cell monolayers (19). Portions of sterile filtrates were incubated at room temperature for 30 min with C. difficile antitoxin (Bureau of Biologics, Rockville, Md.) to test for neutralization. They were also tested for heat lability by heating at 56°C for 30 min. Concentrations of tetracycline in cecal contents were determined by using a modification of the agar diffusion method which was capable of detecting ≥5 μg of tetracycline per ml (20). The minimal inhibitory concentration (MIC) of tetracycline and other antibiotics for C. difficile isolates from hamsters or humans with PMC was determined by broth microdilution methods (10).

RESULTS

Tetracycline-associated enterocolitis. Small amounts of tetracycline (1 and 10 mg/kg)
were nontoxic whether given once or daily (Table 1). Most animals receiving a single high dose (100 mg/kg) of tetracycline orogastrically developed diarrhea, ruffled fur, weight loss, and lethargy (“wet-tail” syndrome) within 3 to 4 days. Death with enterocolitis generally occurred within 8 days of high-dose tetracycline challenge. Hamsters given daily high-dose tetracycline for 7 days or less usually died a few days after it was discontinued, but hamsters given the same amount of tetracycline for more than 7 days died while it was being given (Table 1). Control animals given normal saline remained well.

Postmortem examination of animals that died after receiving tetracycline (100 mg/kg) in either single or daily doses revealed hemorrhagic ceca that were distended with watery, malodorous stools. Histological examination showed acute typhlitis (enterocolitis) with extensive mucosal hemorrhages and focal necrosis; these findings are similar to those with clindamycin (15). Necropsies revealed no macroscopic abnormalities in other organs. Postmortem examinations of sacrificed controls or animals that received lower doses of tetracycline revealed no abnormalities.

The tetracycline-like activity of cecal contents was less than 5 μg/ml 3, 24, or 48 h after a single orogastric administration of 10 mg of tetracycline per kg. After the orogastric administration of a single high dose (100 mg/kg) of tetracycline the levels 3, 24, and 48 h later were 24, 17.5, and <5 μg/ml, respectively.

Protection against clindamycin enterocolitis with tetracycline. All hamsters receiving a single administration of clindamycin (10 mg/kg) orogastrically followed by prophylaxis with daily oral saline developed the wet-tail syndrome and died within a week (mean survival, 3.7 days). Daily orogastric administration of 10 mg of tetracycline per kg after clindamycin challenge did not alter these events (Table 2).

### Table 1. Effect of oral tetracycline

<table>
<thead>
<tr>
<th>Conc of tetracycline administered (mg/kg)</th>
<th>No. of days given</th>
<th>Mortality (no. dead/no. tested)</th>
<th>Mean survival ± SE (days)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single challenge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>0/8</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>0/8</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>1</td>
<td>5/7</td>
<td>8.0 ± 1.7</td>
</tr>
<tr>
<td>Daily challenge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>3</td>
<td>8/9</td>
<td>5.3 ± 0.8</td>
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<tr>
<td></td>
<td>7</td>
<td>6/9</td>
<td>9.7 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>9/10</td>
<td>11.0 ± 0.9</td>
</tr>
</tbody>
</table>

* Number of days after first administration of tetracycline, with exclusion of survivors. SE, Standard error.

### Table 2. Protection by oral tetracycline against clindamycin challenge

<table>
<thead>
<tr>
<th>Drug given daily</th>
<th>Mortality (no. dead/no. tested)</th>
<th>Mean survival after clindamycin ± SE (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clindamycin (10 mg/kg) administered once orogastrically.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9% NaCl for 7 days</td>
<td>14/14</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td>Tetracycline (10 mg/kg) for 7 days</td>
<td>10/10</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>Tetracycline (100 mg/kg)</td>
<td>3 days</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>7 days</td>
<td>12/12</td>
</tr>
<tr>
<td></td>
<td>14 days</td>
<td>13/13</td>
</tr>
</tbody>
</table>

In contrast, animals receiving a larger amount of daily orogastric tetracycline (100 mg/kg) for 3 or 7 days after clindamycin remained well during treatment. However, all of them died of typhlitis a few days after discontinuation of tetracycline (mean survival, 6.6 and 9.5 days after giving clindamycin, respectively). Hamsters that were scheduled to receive tetracycline (100 mg/kg daily) for 14 days after receiving clindamycin appeared well for the first week of treatment, but deaths with enterocolitis occurred sporadically thereafter even during continued tetracycline therapy, and all animals were dead by day 16 post-clindamycin. Thus, all of 35 animals receiving both daily tetracycline and clindamycin (once) died of enterocolitis (Table 2), but only 23 of 28 (82%) hamsters receiving only daily tetracycline died (Table 1).

Gross histopathological examination of animals dying after receiving both clindamycin and tetracycline showed acute typhlitis like that induced with clindamycin alone.

**Presence of toxin in stools.** Sterile filtrates of cecal contents obtained from 28 animals dying after tetracycline were toxic for cell monolayers. A 0.1-ml portion of these filtrates injected intraperitoneally into normal hamsters caused death within 4 to 24 h with evidence of mesenteric fat hemorrhages and pleural effusions. These filtrates were nontoxic after incubation with *C. sordellii* antitoxin and were heat-labile (inactivated by 56°C for 30 min). A total of 46 specimens of filtered cecal contents from hamsters dying after challenge with clindamycin followed by treatment with either saline or tetracycline contained the same type of toxin as those receiving tetracycline alone, as demonstrated by inoculation into hamsters or cell monolayers.

**Bacteriology of stools.** Although we were unable to isolate *S. aureus* from cecal contents of 24 hamsters dying of enterocolitis after treatment with tetracycline (100 mg/kg), *C. difficile* was cultured from ceca of 3 of 5 hamsters with...
toxin-positive enterocolitis that were studied during or after termination of daily tetracycline treatment. When these isolates were incubated anaerobically for 48 h in brain heart infusion broth, sterile filtrates of culture supernatants from all three isolates were toxic for cell monolayers, and their toxicity was neutralized with C. sordellii antitoxin. These isolates were resistant to tetracycline (MIC, 25 to 50 μg/ml) but susceptible to clindamycin (MIC, ≤0.2 to 0.8 μg/ml). In contrast, in our earlier studies the median MIC of tetracycline for 15 C. difficile isolates obtained from hamsters and humans that had not received tetracycline was only 6.25 μg/ml (range ≤0.2 to 50 μg/ml). The median MIC of clindamycin for these isolates was 25 μg/ml (range 0.2 to >100 μg/ml).

DISCUSSION

Tetracycline was reported by Cosar and Kolsky to cause fulminant diarrhea and death in hamsters, but the etiology of the enterotoxic complication was not established (7). In our study the orogastric administration of tetracycline (100 mg/kg) either once or daily caused colitis and death in 28 of 35 hamsters (80%). The lesions resembled those induced by clindamycin that have been attributed to the toxin of C. difficile (4, 15). Cecal contents of 28 moribund or dead animals after tetracycline contained a toxic substance identical in our assays to the toxin elaborated in vitro by C. difficile (2, 4, 19). Whereas we were unable to culture S. aureus from cecal contents of tetracycline-treated hamsters, we were able to isolate C. difficile from three of five animals studied bacteriologically. Since this organism is difficult to isolate, it is not surprising that we did not isolate it from every animal tested. The MIC of tetracycline for inhibition of these isolates of C. difficile was high. This suggested that a possible mechanism of disease production may be by selection of tetracycline-resistant subpopulations. In addition, the C. difficile isolates we obtained from hamsters with tetracycline-induced colitis were susceptible to clindamycin. These data indicate that toxigenic C. difficile are probably responsible for tetracycline-induced colitis in hamsters, as they are for clindamycin-induced colitis in hamsters and humans (4, 5, 18). However, in a recent study by Bartlett et al., oral tetracycline (15 mg/day or about 185 mg/kg per day in three divided doses) was well tolerated (2). These differences may be attributable to their different dosage regimen or may be because their animals were not colonized with C. difficile resistant to tetracycline.

In experiments on clindamycin-induced colitis, co-treatment with tetracycline appeared to postpone but not prevent colitis and death in most animals. Fatal colitis was sometimes observed during the period of tetracycline administration. Although our isolates of C. difficile from human and hamster stools in earlier studies had variable susceptibility to tetracycline (MIC, ≤0.2 to 50 μg/ml), the three isolates from tetracycline-treated hamsters showed uniform resistance to this antibiotic (MIC, 25 to 50 μg/ml). It appeared likely, therefore, that the tetracycline concentrations we achieved in hamster gut (17.5 to 24 μg/ml) were not sufficient to prevent the emergence of organisms resistant to this antibiotic after suppression of normal flora. Administration of small amounts of tetracycline (1 and 10 mg/kg) neither induced the disease nor protected hamsters against clindamycin-associated colitis. Perhaps the levels of tetracycline reached in the gut with these doses (<5 μg/ml) did not alter the flora enough to trigger the overgrowth of the toxigenic organism.

S. aureus-induced PMC associated with tetracyclines was observed in the early antibiotic era (1, 8). The present study suggests that C. difficile also may be capable of causing tetracycline-associated PMC in humans, and such a case of PMC with high titer of neutralized fecal toxin has been reported recently (11). Although DeJesus et al. successfully treated antibiotic-associated diarrhea with tetracycline, stools from patients in their study were not tested for C. difficile toxin; furthermore, it is not known whether their patients had colitis or nonspecific diarrhea. The fact that we obtained tetracycline-resistant strains of C. difficile from hamsters suggests that tetracycline may not be a reliable antimicrobial agent for treatment of antibiotic-associated colitis induced by C. difficile in humans.

Recent reports of treatment of patients with antibiotic-associated colitis with vancomycin suggest it is efficacious. Our C. difficile isolates have been uniformly susceptible to vancomycin, and hamsters can be protected from clindamycin-associated colitis with vancomycin. Thus, based on human and hamster data, vancomycin given orally appears to be effective in treatment of this disease and at present is preferred to tetracycline for treatment of humans.

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LITERATURE CITED


