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In Vitro and In Vivo Evaluation of Tiacumicins B and C against *Clostridium difficile*

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Tiacumicins B and C are members of a novel group of 18-membered macrolide antibiotics with in vitro activity against *Clostridium difficile*. The MICs against 15 strains of *C. difficile* were 0.12 to 0.25 μg/ml for tiacumicin B, 0.25 to 1 μg/ml for tiacumicin C, and 0.5 to 1 μg/ml for vancomycin. The resistance frequency for both compounds against *C. difficile* was $<2.8 \times 10^{-8}$ at four and eight times the MIC. The in vivo activities of the tiacumicins against two strains of *C. difficile* were compared with that of vancomycin in a hamster model of antibiotic-associated colitis. Oral therapy with 0.2, 1, or 5 mg of tiacumicin B or C per kg of body weight protected 100% of clindamycin-treated hamsters exposed to *C. difficile* ATCC 9689. Oral treatment with identical doses of vancomycin produced a prolonged, dose-dependent survival of hamsters, but it did not prevent the development of fatal colitis at doses of up to 5 mg/kg. When clindamycin-treated animals were exposed to another strain of *C. difficile*, both tiacumicin B and vancomycin were protective at 5 mg/kg, but not at lower doses. Tiacumicin C was not tested in vivo against the second strain of *C. difficile*. No tiacumicin B or C was detected in the sera of hamsters treated with single oral doses of 25 mg/kg, while antibiotic levels in the ceca of these hamsters reached 248 μg/ml and 285 mg/ml for tiacumicins B and C, respectively. The tiacumicins demonstrated in vitro and in vivo potencies against *C. difficile* and achieved high concentrations in the cecum, but not the serum, of hamsters after oral administration.

The tiacumicins are a group of 18-membered macrolide antibiotics originally isolated from the fermentation broth of *Dactylosporangium aurantiacum* subsp. *hamdenensis* (7, 16). Tiacumicin B, the major antibiotic component produced by this culture, contains an unsaturated 18-membered macrolide ring with a seven-carbon sugar at carbon 11 and a 6-deoxy sugar at carbon 20 (Fig. 1a). This compound is apparently identical to one of the lipiarmycins, a previously described group of antibiotics produced by *Actinoplanes deccanensis* (1, 10, 12), and to clostomicin B1, an antibiotic from *Micromonospora echinospora* (9). Tiacumicin C differs in the position of butyrate esterification on the seven-carbon sugar (Fig. 1b). Tiacumicin C appears to be identical to clostomicin B2, a member of the clostomicin complex isolated from *M. echinospora* (9).

A preliminary report on the biological properties of the tiacumicins indicated that tiacumicin B has moderate activity against pathogenic strains of *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Enterococcus faecium* and demonstrated that it has limited, but potent, activity against several anaerobic bacteria (16). In the same study, tiacumicin C was found to have a similar spectrum of activity but to be less potent than tiacumicin B.

The present study was designed to examine the in vitro activities of the tiacumicins against *Clostridium difficile* and to evaluate the efficacies of these compounds in treating antibiotic-associated pseudomembranous colitis caused by this bacterium. Pharmacokinetic data from hamsters orally treated with the tiacumicins are also presented.

(Part of this research was presented at the 28th Interscience Conference on Antimicrobial Agents and Chemotherapy [14].)

MATERIALS AND METHODS

Bacterial strains. The strains used in this study were from the Abbott Laboratories (Abbott Park, Ill.) culture collection, American Type Culture Collection, and Virginia Polytechnic Institute. *C. difficile* CD630 was obtained from Herbert Hachler (Department of Microbiology, University of Zurich) and has genetically characterized erythromycin-clindamycin resistance (6). All strains were maintained at −60°C.

Antibacterial agents. Tiacumicins B and C were prepared at Abbott Laboratories. Vancomycin (Eli Lilly & Co., Indianapolis, Ind.), clindamycin (The Upjohn Co., Kalamazoo, Mich.), and metronidazole (G. D. Searle and Co., Skokie, Ill.) were purchased from their respective manufacturers. All compounds were prepared in sterile injectable water immediately before administration to animals.

In vitro potency. MICs in Wilkins-Chalgren agar were determined by a standard twofold agar dilution procedure (13).

Effect of serum and pH on in vitro activity. The effects of 50% hamster serum and at pHs 6.5, 7.3, and 8.0 on the in vitro potencies of the tiacumicins in Wilkins-Chalgren broth were determined by a microdilution method (13). To separate the effect of pH from the effect of serum, the pHs of the serum samples were adjusted to 7.3 prior to testing.

Effect of hamster cecal contents on in vitro activity. The bioavailabilities of the tiacumicins in the presence of normal hamster cecal contents were determined by a previously described methodology (13). Briefly, MICs and MBCs of

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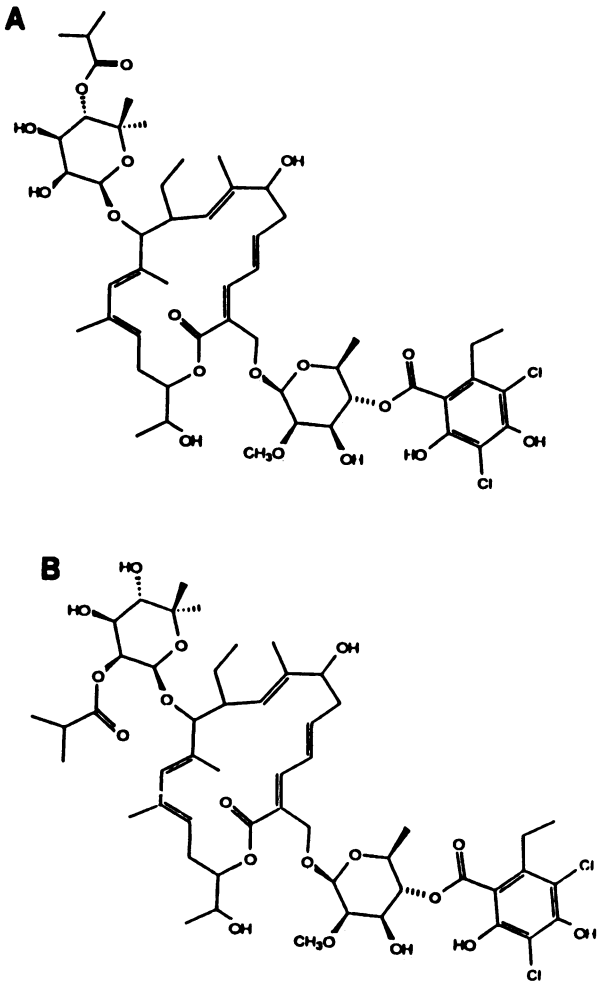

FIG. 1. Chemical structures of tiacumicin B (A) and tiacumicin C (B).

tiacumicins B and C in Wilkins-Chalgren broth and in broth containing a 5% suspension of filtered cecal contents were compared.

Frequency of resistance. The frequency of spontaneous resistance development by *C. difficile* ATCC 9689 exposed to four and eight times the MICs of tiacumicin B, tiacumicin C, vancomycin, and metronidazole in Wilkins-Chalgren agar was determined by a previously described method (4). In brief, an isolated colony inoculated into Wilkins-Chalgren broth was incubated anaerobically at 37°C to the logarithmic phase of growth. Viable cell counts in this broth culture were determined by spreading 0.1 ml of appropriate dilutions in triplicate onto drug-free Wilkins-Chalgren agar plates. Ten 0.1-ml samples of the undiluted culture were plated by spreading them onto 10 plates containing four or eight times the MIC of a compound; this procedure was repeated for each of the test compounds. All plates were incubated anaerobically at 37°C for a total of 42 h. After incubation, the number of colonies on all agar plates was counted; the cell density in the initial inoculum was calculated from colony counts on drug-free agar. The number of resistant colonies on drug-containing plates was expressed as a fraction of the total number of cells per milliliter.

Hamster model for pseudomembranous colitis. Male Golden Syrian hamsters (weight, 80 to 100 g) were purchased from Harlan Sprague Dawley, Inc., Indianapolis, Ind. The hamsters were caged in groups of five and were given Purina rodent chow and water ad libitum. Antibiotic-associated colitis was induced in hamsters by a modification of previously reported techniques (2, 13). After a quarantine period of 7 days, the hamsters received 100 mg of clindamycin per kg of body weight intraperitoneally. Twenty-four hours later, the hamsters were orally challenged with 10^6 *C. difficile* ATCC 9689 organisms. The hamsters then were randomized to groups of eight per cage. Beginning 8 h after infection, hamsters received therapy with 0.2, 1, or 5 mg of vancomycin, tiacumicin B, or tiacumicin C per kg. All antibiotics were administered orally once a day for 5 days. Eight animals (two cages holding four hamsters each) were used in each treatment group. The therapeutic regimens for the different groups were run concurrently, and all animals within each cage received identical treatment. The cecal contents were removed from hamsters that died during the experiment and were analyzed for *C. difficile* antigens by a rapid slide agglutination test (Marion Laboratories, Kansas City, Mo.). An increase in this antigen has been associated with clinical disease in humans (8). The hamsters were monitored for 35 days, and the cumulative mortality during this period was recorded.

Minor modifications were made in the procedure described above to induce colitis with a second strain, *C. difficile* 2926. Hamsters were orally inoculated with approximately 10^6 bacteria 24 h after intraperitoneal treatment with 30 mg of clindamycin per kg. Beginning 8 h after infection, groups of five hamsters each received oral therapy with 1 or 5 mg of tiacumicin B or vancomycin per kg. Tiacumicin C was not available in sufficient quantities for use in this test. The compounds were administered once daily for 5 days. Drug efficacy was evaluated as described above. *C. difficile* antigen testing was performed with cecal contents removed from only control hamsters that died 2 days postinfection.

Pharmacokinetic studies in hamsters. Male Golden Syrian hamsters (weight, 80 to 100 g) were administered 25 mg of tiacumicins B and C per kg orally. At 1, 2, 3, 6, 8, and 24 h after treatment, blood and cecal contents were collected from groups of two hamsters. Samples were assayed for antibiotic by high-pressure liquid chromatography.

Acute toxicities in mice. Acute toxicities were determined for both the tiacumicins by using female CF-1 mice (weight, 18 to 20 g). Groups of five mice each were injected intraperitoneally at four dose levels for each antibiotic. Each dose was administered as a single injection. The 50% lethal doses were calculated from the cumulative mortalities on day 5 after inoculation by the method of Reed and Muench (11).

RESULTS

In vitro activity. The MICs of tiacumicins B, C, and reference compounds for *C. difficile* are presented in Table 1. Tiacumicin B was two- to fourfold more potent than tiacumicin C and vancomycin against *C. difficile* and was at least as active as metronidazole against these strains. Both tiacumicins demonstrated activity against *C. difficile* strains that expressed high-level resistance to clindamycin.

Effect of pH and serum on in vitro activity. The MICs of tiacumicins B and C and vancomycin for *C. difficile* ATCC 9689 determined at pHs 6.5 and 8.0 were unchanged or only twofold different from the MICs determined at pH 7.3. The MICs of tiacumicins B and C and vancomycin for *C. difficile* ATCC 9689 determined in 50% hamster serum were in-

TABLE 1. Activities of tiacumicins B and C, vancomycin, clindamycin, and metronidazole against *C. difficile*

Compound	MIC (μg/ml)[a]		
	Range	50%	90%
Tiacumicin B	0.12–0.25	0.25	0.25
Tiacumicin C	0.25–1	0.5	1
Vancomycin	0.5–1	0.5	1
Clindamycin	1–>128	8	>128
Metronidazole	0.12–0.5	0.25	0.5

[a] 50% and 90%, MIC for 50 and 90% of isolates, respectively.

creased 64-, 32-, and 8-fold, respectively, compared with the MICs determined in broth at pH 7.3 without serum.

Bioavailability in cecal contents. Both tiacumicins B and C were less active against *C. difficile* ATCC 9689 in the presence of hamster cecal contents. The MIC of tiacumicin B increased from <0.015 μg/ml in Wilkins-Chalgren broth to 2 μg/ml in broth containing 5% cecal contents. The MIC of tiacumicin C increased from 0.12 μg/ml in broth to 4 μg/ml in broth containing cecal contents. The MBCs of tiacumicins B and C in broth and broth supplemented with cecal contents were equal to their respective MICs.

Frequency of resistance. The frequency of spontaneous resistance development of *C. difficile* ATCC 9689 to four and eight times the MICs of tiacumicins B and C, vancomycin, and metronidazole was $<2.8 \times 10^{-8}$.

In vivo activity. After treatment with clindamycin and exposure to *C. difficile* ATCC 9689, 75% of the control hamsters died within 10 days (Fig. 2). Two of eight control hamsters remained alive throughout the 35-day observation period. All moribund hamsters had diarrhea prior to death. Upon necropsy these animals had distended, fluid-filled ceca. *C. difficile* antigens were detected in the cecal contents from all the control hamsters that died throughout the experiment (3 to 10 days postinfection). Vancomycin therapy protected the hamsters in a dose-dependent manner. Fatal colitis developed in 100% of the hamsters that received 0.2 mg of vancomycin per kg, whereas fatal colitis developed in 87.5% of the hamsters given 1 mg of vancomycin per kg and in 62.5% of hamsters given 5 mg of vancomycin per kg.

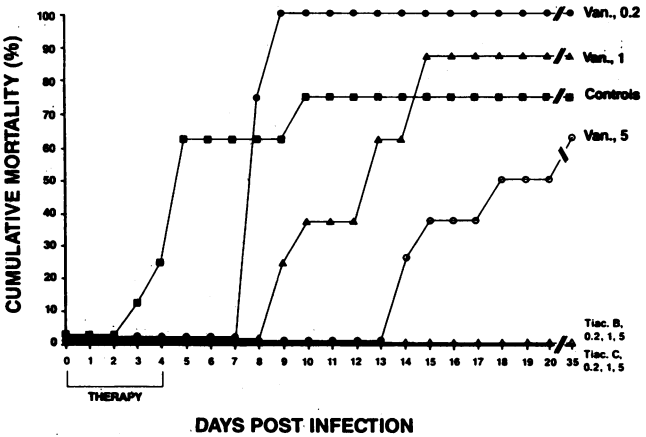

FIG. 2. Cumulative mortalities caused by clindamycin-induced *C. difficile* ATCC 9689 colitis in hamsters that received oral therapy with tiacumicin B (Tiac. B), tiacumicin C (Tiac. C), or vancomycin (Van.) at 0.2, 1, and 5 mg/kg.

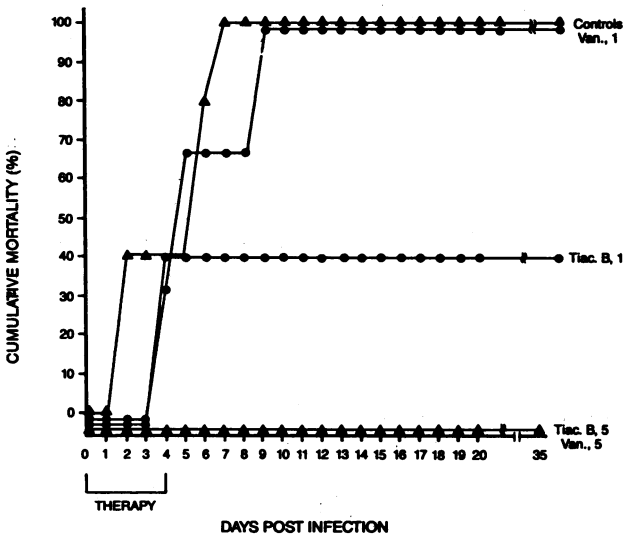

FIG. 3. Cumulative mortalities caused by clindamycin-induced *C. difficile* 2926 colitis in hamsters that received oral therapy with tiacumicin B (Tiac. B) or vancomycin (Van.) at 1 and 5 mg/kg.

C. difficile antigens were detected in the cecal contents of vancomycin-treated hamsters that died 8 to 21 days postinfection. The mean time to death was 8.25, 12, and 16 days in hamsters treated with 0.2, 1, and 5 mg of vancomycin per kg, respectively. All hamsters that received tiacumicins B or C at 0.2, 1, or 5 mg/kg survived for at least 35 days after clindamycin treatment. Therefore, oral therapy with tiacumicins B or C at doses as low as 0.2 mg/kg completely prevented the development of antibiotic-induced *C. difficile* colitis in hamsters.

In a second experiment, fatal colitis was induced in clindamycin-treated hamsters exposed to *C. difficile* 2926. One hundred percent of control hamsters died within 7 days postinfection (Fig. 3). Mortality rates in hamsters treated with 1 and 5 mg of vancomycin per kg were 100 and 0%, respectively. The mean time to death in hamsters treated with 1 mg of vancomycin per kg was 6 days. This compared with mortality rates of 40 and 0% in hamsters treated with 1 and 5 mg of tiacumicin B per kg, respectively. The mean time to death was 7 days in hamsters that received 1 mg of tiacumicin B per kg. *C. difficile* antigens were detected in both control animals that died 2 days after infection. No additional antigen testing was conducted in this experiment.

Pharmacokinetic studies. No tiacumicin B or C was detected in the serum of hamsters treated with a single oral dose of 25 mg/kg. Levels of tiacumicin B in the cecal contents of these animals were 137, 188, 139, 248, 98, and 58 μg/g at 1, 2, 3, 6, 8, and 24 h after administration, respectively. Levels of tiacumicin C in cecal contents were 90, 285, 255, 55, 97, and 101 μg/g at 1, 2, 3, 6, 8, and 24 h, respectively.

Toxicity tests. The 50% lethal doses in intraperitoneally injected mice were >500 mg/kg for tiacumicin B and 294 mg/kg for tiacumicin C.

DISCUSSION

The tiacumicins are 18-membered macrolide antibiotics, some of which have activity against pathogenic gram-positive bacteria and selected anaerobes (16). When tested in vitro against 15 strains of *C. difficile* in the present study,

tiacumicin B was at least as potent as metronidazole and was two- to fourfold more active than vancomycin. Tiacumicin C was as potent as vancomycin against *C. difficile*. The potent activity demonstrated by the tiacumicins against these bacteria led us to test these compounds in an animal model of pseudomembranous colitis caused by *C. difficile*. In this model, both tiacumicins B and C were considerably more effective than vancomycin in treating antibiotic-induced colitis caused by *C. difficile* ATCC 9689 and in preventing recurrence after therapy was discontinued. Treatment with tiacumicin doses as low as 0.2 mg/kg prevented the development of fatal colitis in clindamycin-treated hamsters. Vancomycin, on the other hand, delayed but did not prevent the development of fatal colitis. This is in agreement with results of other studies in hamsters that indicate that vancomycin prevents colonization with *C. difficile* while it is being administered but fails to correct the predisposing conditions within the gut that allow this bacterium to later cause disease (3). In treating antibiotic-induced colitis caused by *C. difficile* 2926, tiacumicin B was slightly more effective than vancomycin when administered orally at 1 mg/kg (40% survival in tiacumicin B-treated animals compared with 0% survival in vancomycin-treated animals). At 1 mg/kg, both tiacumicin B and vancomycin were more effective in treating colitis caused by *C. difficile* ATCC 9689 than that caused by *C. difficile* 2926. This may have been due in part to the increased susceptibility of *C. difficile* ATCC 9689 to tiacumicin B (the MIC of tiacumicin B was twofold lower against the ATCC 9689 strain) or to the altered susceptibilities of these *C. difficile* strains to clindamycin, the inducing antibiotic. The MICs of clindamycin were 4 μg/ml against *C. difficile* ATCC 9689 and >128 μg/ml against *C. difficile* 2926. It is also possible that the different levels of efficacy were due to altered methodologies. *C. difficile* ATCC 9689 colitis was induced with 100 mg of clindamycin per kg, while *C. difficile* 2926 colitis was induced with 30 mg of clindamycin per kg. The effects of various clindamycin doses on the severity of antibiotic-induced colitis in hamsters have not been determined.

Several properties of the tiacumicins may account for their ability to completely prevent *C. difficile* colitis in clindamycin-treated hamsters. Both tiacumicins are very potent in vitro against *C. difficile* and are bactericidal on the basis of their MBCs in broth and broth supplemented with cecal contents. Because they are bactericidal, the tiacumicins may be effective in eradicating *C. difficile* from the intestinal tract by preventing recolonization of this site after use of the antimicrobial agent has been discontinued. In addition, the tiacumicins accumulate to high concentrations in the ceca of hamsters treated orally with these agents. The absence of tiacumicins in the sera of treated animals suggests that the tiacumicins are not adsorbed systemically and remain concentrated at the site of infection.

Pseudomembranous colitis is a serious condition which can occur in patients following treatment with a wide range of antibiotics, including clindamycin, ampicillin, cephalosporins, and others (5). The drugs of choice for treating this condition are vancomycin and metronidazole, both of which have limitations. Included among these limitations is the fact that both vancomycin and metronidazole treatments result in unacceptably high relapse rates (5, 15). The tiacumicins, because of their potent anti-*C. difficile* activities and their ability to achieve high concentrations in the gut, should be further studied as potential therapeutic agents in treating pseudomembranous colitis. The tiacumicins compare favorably to LY 146032, a lipopeptide antibiotic previously reported to delay but not prevent *C. difficile* colitis in clindamycin-treated hamsters (2).

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