In Vitro and In Vivo Activities of Sedecamycin against Treponema hyodysenteriae

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Sedecamycin (lankacidin A), one of the lankacidin-group antibiotics, showed potent activity against Treponema hyodysenteriae. The MICs of sedecamycin against 79 field isolates of T. hyodysenteriae ranged from 0.78 to 12.5 μg/ml, the MIC for 90% of the strains tested (MIC90) being 3.13 μg/ml. The protective and therapeutic effects of sedecamycin were compared with those of carbadox, tiamulin, and lincomycin against experimental infection with T. hyodysenteriae in mice. The protective effect of sedecamycin was similar to that of carbadox, two times more potent than that of tiamulin, and three times greater than that of lincomycin. In the therapeutic test, sedecamycin showed activity similar to that of carbadox and was two times more active than both tiamulin and lincomycin. At doses of 10 mg or more of sedecamycin per kg, the recurrence of shedding of T. hyodysenteriae into the feces of mice was not detected for at least 8 weeks postmedication.

Lankacidin-group antibiotics isolated from the culture filtrate of Streptomyces rochei var. volubilis (3, 5, 7, 11) have a characteristic 17-membered macrocyclic structure (4-6, 13). They show strong antibacterial activity mainly against gram-positive bacteria (8, 11, 27). In the serial screening procedure in these laboratories, some of the antibiotics were found to be active against Treponema hyodysenteriae, an etiologic agent for swine dysentery (10, 17, 26). Sedecamycin (the generic name of lankacidin A) (Fig. 1) was selected from the candidates because it showed excellent activity against T. hyodysenteriae in vitro and provided good efficacy against T. hyodysenteriae infection in mice and pigs (T. Yamazaki, N. Narukawa, I. Suenaga, and K. Takeda, Proc. 9th Congr. Int. Pig Vet. Soc., Spain, p. 178, 1986). Sedecamycin is marketed as a feed additive drug for the treatment of swine dysentery in Japan. This report describes the in vitro antimicrobial activities of sedecamycin against T. hyodysenteriae and the efficacy of the drug in experimental infection with T. hyodysenteriae in mice.

MATERIALS AND METHODS

Antimicrobial agents. Sedecamycin was prepared by Takeda Chemical Industries, Ltd. (Osaka, Japan). Carbadox (Pfizer Taito Co., Ltd., Tokyo, Japan), tiamulin (Squibb Japan Inc., Tokyo, Japan), and lincomycin (Sigma Chemical Co., St. Louis, Mo.) were used as reference drugs.

Microorganisms. T. hyodysenteriae CD1, DJ70P1, and 78/A were kindly supplied by M. Kashiwazaki, National Institute of Animal Health, Ibaraki, Japan. After two passages of DJ70P1 through swine, isolated T. hyodysenteriae from swine are denoted as DJ70P3. A total of 79 clinical isolates of T. hyodysenteriae were obtained from various pig farms around Japan. The isolated organisms were identified as follows: (i) growth with typical beta-hemolytic zone around colonies on Trypticase soy agar (TSA; BBL Microbiology Systems, Cockeysville, Md.) supplemented with 400 μg of spectinomycin per ml and 5% horse blood after incubation at 37°C for 4 days in an anaerobic GasPak jar (BBL) (23); (ii) when viewed by phase-contrast microscopy, the large spirochete was a motile, loosely coiled, and spiral organism (10). T. hyodysenteriae was grown anaerobically at 37°C for 4 days in a GasPak jar on blood-TSA. The beta-hemolytic zone of this plate was cut into a block (3 by 8 by 10 mm) and kept in a plastic tube at −80°C.

Determination of MIC. MICs of the compounds were determined by the agar dilution method with blood-TSA. The inoculum was prepared as follows. A frozen block (3 by 8 by 10 mm) of blood-TSA in which T. hyodysenteriae had been grown was transferred to and allowed to thaw on a blood-TSA plate. Then, the plate was incubated anaerobically at 37°C for 4 days in a GasPak jar. At the end of the incubation period, a block (3 by 8 by 20 mm) was cut from the peripheral part of the beta-hemolytic zone of the blood-TSA and blended with 2 ml of Trypticase soy broth (TSB; BBL), using a Touch Mixer (Yamato Scientific, Tokyo, Japan). Inocula of 5 μl of diluted treponemal suspensions of approximately 10³ CFU/ml were applied to blood-TSA plates with a multipoint replicating apparatus (Sakuma Sei-sakusho, Tokyo, Japan). The plates were incubated at 37°C for 48 h in a GasPak jar, and the MIC was defined as the lowest concentration of antitreponemal agent that completely prevented hemolysis (14). Using the same treponemal suspension, the broth dilution MIC was determined with TSB containing 10% fetal calf serum (15). Tubes were incubated at 42°C for 48 h in an anaerobic glove box (Tabai Espec, Osaka, Japan) containing 5% hydrogen and 25% carbon dioxide in nitrogen, and the MIC was defined as the lowest concentration of drug that did not permit any visible growth. Specimens of T. hyodysenteriae for scanning electron microscopy were prepared from the culture broth used for MIC determination by the method described by Nakao et al. (18).

Experimental infection in mice. Four-week-old Ta:CF#1 female mice (Takeda Chemical Industries, Ltd.) (25) were used in this study. The challenge inoculum of T. hyodysenteriae DJ70P3 was prepared by the method described previously (24). Mice were inoculated orally with 0.5 ml of an inoculum containing approximately 10⁶ CFU of T. hyodysen-

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The protective effect of wood shavings infected with T. hyodysenteriae was repeated groups isolate of T. hyodysenteriae orally once a day starting 4 days before inoculation. Blood-TSA containing 400 µg of spectinomycin per ml (23) was used to isolate T. hyodysenteriae from the cecum selectively. In this experiment, groups of five mice each were used and the test was repeated several times.

Therapeutic test. Drugs were administered orally to mice infected with T. hyodysenteriae once a day for 4 days starting 7 days after inoculation, at which time fecal and cecal counts of the organisms reach a high level (10⁷ to 10⁸ CFU/g) and gross cecal lesions are observed in more than half of the mice (25). The mice were necropsied on postinoculation day 14 to investigate the colonization of T. hyodysenteriae in the cecum. Groups of five mice each were used in this experiment.

Recurrence test. Mice infected with T. hyodysenteriae were given sedecamycin once a day for 4 days beginning on postinoculation day 7 and were kept for an 8-week postmedication period. A fecal specimen was collected from each mouse on postmedication day 1 and then on day 7 and every 7 days during the postmedication period; this was cultured to test for the presence of T. hyodysenteriae. The mice were necropsied after the postmedication period to monitor colonization by T. hyodysenteriae in the cecum. The bedding of wood shavings was changed weekly.

RESULTS

In vitro activity. (i) Agar dilution MIC. The activity of sedecamycin against four laboratory strains of T. hyodysenteriae was compared with that of carbadox, tiamulin, and lincomycin. The MICs of the antibiotics were as follows: sedecamycin, 3.13 µg/ml (strain 78/A) and 6.25 µg/ml (strains DJ70P1, DJ70P3, and DJ70P3); carbadox, 0.006 µg/ml (strains DJ70P1, DJ70P3, and 78/A) and 0.013 µg/ml (strain DJ1); tiamulin, 0.1 µg/ml (four strains); and lincomycin, 0.013 µg/ml (four strains). The MICs of sedecamycin were lower than those of lincomycin and higher than those of carbadox and tiamulin.

The MICs of sedecamycin against 79 field isolates of T. hyodysenteriae ranged from 0.78 to 12.5 µg/ml, the MIC for 90% of the strains tested (MIC₉₀) being 3.13 µg/ml. The MIC₉₀s of carbadox, tiamulin, and lincomycin for T. hyodysenteriae were 0.006 µg/ml (range, 0.003 to 0.013 µg/ml), 0.2 µg/ml (0.025 to 0.2 µg/ml), and 50 µg/ml (0.2 to 100 µg/ml), respectively.

(ii) Broth dilution MIC. The broth dilution MICs of sedecamycin for T. hyodysenteriae DJ70P3 and 78/A were 6.25 and 3.13 µg/ml, respectively, as compared with 0.013 and 0.006 µg/ml, respectively, for carbadox, 0.2 and 0.1 µg/ml, respectively, for tiamulin, and 50 µg/ml (both strains) for lincomycin.

Scanning electron micrographs of T. hyodysenteriae 78/A exposed to sedecamycin for 48 h are shown in Fig. 2. Sedecamycin induced marked morphological changes at the MIC (3.13 µg/ml) and above the MIC (data not shown), comprising swelling, lysis of treponemes, and the attachment of fibrillar material to the organisms.

In vivo activity. (i) Protective effect. The protective effect of sedecamycin in mice infected with T. hyodysenteriae DJ70P3 was compared with that of carbadox, tiamulin, and lincomycin. Sedecamycin showed a strong protective effect, the ED₅₀ being 6.4 mg/kg and the 95% confidence limits being 5.6 to 7.7 mg/kg. The ED₅₀s of carbadox, tiamulin, and lincomycin were 7.7 mg/kg (95% confidence limits, 6.2 to 9.6 mg/kg), 14.6 mg/kg (11.9 to 18.0 mg/kg), and 21.7 mg/kg (16.8 to 28.2 mg/kg), respectively. Sedecamycin was as active as carbadox, two times more effective than tiamulin (P < 0.05), and three times more effective than lincomycin (P < 0.05).

(ii) Therapeutic effect. Sedecamycin given therapeutically in mice infected with T. hyodysenteriae was highly effective, the ED₅₀ being 6.4 mg/kg and the 95% confidence limits being 5.6 to 7.7 mg/kg.
TABLE 1. Recurrence of shedding of *T. hyodysenteriae* into the feces of mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose* (mg/kg)</th>
<th>No. of <em>T. hyodysenteriae</em>-positive mice*</th>
<th>Premedication</th>
<th>1 day</th>
<th>1 wk</th>
<th>2 wk</th>
<th>3 wk</th>
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<th>5 wk</th>
<th>6 wk</th>
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<tr>
<td>Sedecamycin</td>
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<td>5</td>
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<td>2</td>
<td>4</td>
<td>5</td>
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* Sedecamycin was administered orally daily for 4 days starting on day 7 after inoculation.
* Five mice were tested in each dosage group. Time indicated is time after the completion of medication.

being 3.6 to 11.1 mg/kg. The ED₅₀ of carbadox, tiamulin, and lincomycin were 5.0 mg/kg (95% confidence limits, 2.4 to 8.7 mg/kg), 12.1 mg/kg (6.9 to 21.6 mg/kg), and 12.8 mg/kg (7.3 to 22.1 mg/kg), respectively. Sedecamycin was as active as carbadox and two times more effective than both tiamulin and lincomycin.

(iii) Recurrence. The recurrence of shedding of *T. hyodysenteriae* into the feces of mice on postinoculation days 7 to 10 after therapeutic treatment with sedecamycin is shown in Table 1. In mice treated with sedecamycin at a dose of 5 mg/kg, *T. hyodysenteriae* was not detected on postmedication day 1. However, from 1 week after medication, the number of mice shedding the organisms gradually increased and the mice shed the bacteria from the postmedication week 3 to 8. At doses of 10 mg or more of sedecamycin per kg, shedding of *T. hyodysenteriae* was not detected in any mouse during the postmedication period.

**DISCUSSION**

Sedecamycin was found to be highly active against *T. hyodysenteriae*. In vitro, sedecamycin was superior to lincomycin but less active than carbadox and tiamulin. The MICs found in this study for these three reference compounds were comparable to those reported by Kitai et al. (14).

Although the antitreponemal activity of sedecamycin was less than that of carbadox and tiamulin in vitro, sedecamycin showed more potent activity than tiamulin and activity similar to that of carbadox against experimental infection of *T. hyodysenteriae* in mice. One of the essential factors affecting the effectiveness of antitreponemal agents in this model infection appears to be the MIC of the agents in the cecum and their duration of action. The activity of drugs against anaerobic bacteria, in addition to *T. hyodysenteriae*, may also be a factor, since typical colonic lesions of swine dysentery are induced in gnotobiotic pigs challenged with *T. hyodysenteriae* in combination with certain anaerobes such as *Bacteroides vulgatus*, *Fusobacterium necrophorum*, and *Clostridium* sp. (9, 28). A pathogenic synergism between *T. hyodysenteriae* and an anaerobic bacterium has also been observed in gnotobiotic mice inoculated with *T. hyodysenteriae* in combination with *B. vulgatus* (12). Therefore, the potent activity of sedecamycin in the model infection used may be related to its antimicrobial activity against anaerobes, in addition to its activity against *T. hyodysenteriae*. Studies are in progress on the presence of the pathogenic anaerobes in the ceca of Tła:CF#1 mice that are synergistic for *T. hyodysenteriae*.

Several attempts have been made to eradicate *T. hyodysenteriae* from swine by drugs both in the field and in the laboratory, but a recurrence of bloody scour was observed in the postmedication period; thus, the disease recurs frequently after administration of carbadox (1, 20), lincomycin (2, 21), olaquindox (22), and ronidazole (19). In this study, infected mice were treated with sedecamycin and the incidence of recurrence of shedding of *T. hyodysenteriae* was investigated for 8 weeks after medication. Recurrence was observed in mice given 5 mg of sedecamycin per kg, a dose less than the ED₉₀ (6.4 mg/kg), but not in mice treated with 10 mg or more of sedecamycin per kg. It has been reported that the generation time of *T. hyodysenteriae* is 5 h at 37°C in liquid medium (15). Thus, if any treponemes remained in the cecum subsequent to treatment with sedecamycin, 8 weeks would be an adequate period for reestablishment of the infection. It seems that sedecamycin at 10 mg or more per kg eradicated *T. hyodysenteriae* from the ceca of mice. This is an encouraging result for the application of sedecamycin for control of swine dysentery.

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**LITERATURE CITED**