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Cephamycin C Treatment of Induced Enterotoxigenic Colibacillosis (Scours) in Calves and Piglets

THOMAS M. JACKS,* KLAUS-DIETER SCHLEIM, FREDERICK R. JUDITH, AND BRINTON M. MILLER

Merck Institute for Therapeutic Research, Rahway, New Jersey 07065

Cephamycin C is a β-lactam antibiotic that has broad gram-negative activity and is resistant to degradation by β-lactamases and safe for use in animals. In colostrum-fed calves infected with *Escherichia coli* strain B44, cephamycin C administered by gavage at 31.3 to 1,000 mg per calf (0.75 to 24 mg/kg) twice a day for 6 days starting at 20 h post-inoculation eliminated the diarrhea and reduced the mortality from 90% in infected, nonmedicated calves to 14% in infected, medicated calves ($P < 0.01$). Comparable results were obtained with a shorter treatment regimen (30 mg of cephamycin C per calf [0.71 mg/kg] twice a day for 3 days). In colostrum-fed piglets infected with *E. coli* strain P155 and housed in cages, cephamycin C administered prophylactically by gavage at 12.5 mg per piglet (10.4 mg/kg) twice a day for 4 days completely prevented both diarrhea and mortality, whereas nonmedicated piglets had 100% diarrhea and all died. When eight doses of cephamycin C were given therapeutically starting at 6 h post-inoculation, mortality was reduced from 79 to 23% ($P < 0.02$), and diarrhea was eliminated in the surviving medicated piglets by 4 days post-inoculation. In infected suckling piglets, cephamycin C administered therapeutically by gavage at 12.5 mg per piglet twice a day for 3 days starting at 6 h post-inoculation, diarrhea and mortality were reduced ($P < 0.05$): infected, nonmedicated piglets had 87% diarrhea and 75% mortality, whereas infected, medicated piglets had 25% diarrhea and 31% mortality. All surviving medicated piglets had solid feces by 2 days post-inoculation. Thus, cephamycin C was highly effective in restoring the calves and piglets to good health by eliminating diarrhea and reducing mortality.

Cephamycin C, a β-lactam antibiotic produced by *Streptomyces lactamdurans*, has activity against a broad spectrum of gram-negative organisms in vitro (13) and in vivo (12) and is resistant to degradation by β-lactamases (6) and safe for use in animals. It is currently not used for therapy in animals or humans.

Enterotoxigenic *Escherichia coli* strains (ETEC) cause fatal diarrhea in newborn calves (1, 3, 14, 17) and piglets (15, 16). These ETEC colonize the mucosal surface of the small intestine without tissue invasion, multiply to large numbers, and elaborate an enterotoxin(s) which causes the diarrhea. Attachment to the mucosal surface is mediated by pili designated as K99 on bovine ETEC and designated as K88 or 987P on porcine ETEC.

These ETEC produce two types of enterotoxin, a heat stable low-molecular-weight type (ST) and a heat-labile, high-molecular-weight type (LT) (2, 5). All bovine ETEC described to date produce ST only, whereas porcine ETEC produce either ST only or LT plus ST. These experiments were designed to determine the level of cephamycin C that would be effective in the treatment of induced enteric colibacillosis in dairy calves and piglets.

MATERIALS AND METHODS

Cultures. A nalidixic acid-resistant mutant of bovine ETEC B44 (O9:K30:K99:H−), an ST-only producer, was used in calves (1, 14, 17). A nalidixic acid-resistant mutant of porcine ETEC P155 (O149:K91:K88), an LT plus ST producer, was used in piglets (16). Both strains produce a methanol-soluble ST which is active in infant mice (4). The minimal inhibitory concentration of cephamycin C for each strain is 8 μg/ml.

Challenge infection. All studies were conducted at the Merck research farm in Branchburg Township, N.J. Holstein or Holstein-Angus cross calves less than 15 h old were acquired from local dairy farms. The calves were placed in individual metal cages, four cages to each isolation room. Each calf received 1 liter of colostrum at the first feeding and 2 liters of whole milk twice daily thereafter. Calves were challenged orally with approximately 10^{11} colony-forming units (CFU) of strain B44 (washed cells in 50 ml of saline) in the late afternoon of the day they arrived and always after the first feeding of colostrum. Unchallenged control calves were maintained in separate rooms. The fecal consistency of each calf was scored, and a fecal sample

or rectal swab was collected for culture each morning. Calves were maintained for 10 days, and all calves were necropsied the day they died or at the end of the experiment. Calves were weighed when they were received (weight range was 30 to 52 kg with a mean weight of 42 kg) and at necropsy.

Yorkshire pigs (Willow Grove Farm, Stroudsburg, Pa.) were used. In the caged piglet experiments, piglets were separated from the sow 18 to 24 h after farrowing and moved to an isolation room where they were individually housed in wire cages and fed whole milk 3 times daily. Each litter was distributed into cages according to sex and size. The next morning each piglet was challenged orally with approximately 10^{10} CFU of P155 (washed cells). Unchallenged control piglets were maintained in a separate room.

In the suckling piglet experiments, the piglets were farrowed and kept with the sow in an isolation room throughout the experiment. Twelve to 18 h after farrowing, each piglet in a litter was challenged orally with approximately 10^{10} CFU of P155. At 8 h postinoculation (PI) each litter was divided by sex and size into two equal groups; each piglet in one group was medicated, whereas none of the piglets in the other group was medicated. In both the caged piglet and suckling piglet experiments, the piglets were observed each morning; the fecal consistency of each piglet was scored, and a fecal sample or swab was taken for culture. All piglets were necropsied the day they died or at the end of the experiment (8 to 10 days PI). At the time of inoculation, the weight range was 0.64 to 1.86 kg with a mean weight of 1.20 kg.

Clinical observations and bacteriology. Fecal consistencies of the calves and piglets were scored as follows: 3, profuse watery feces with little solid material; 2, watery feces with some solid material; 1, semisolid feces; 0, solid feces.

Fecal samples from calves were processed immediately after collection. B44 counts were made on homogenized fecal samples by serially diluting 1 g of wet feces with phosphate-saline solution. Appropriate dilutions were plated on Levine EMB agar plates containing 100 μg of nalidixic acid per ml. The plates were incubated for 24 h at 37°C, and then *E. coli* colonies were enumerated. Fecal swabs from piglets were qualitatively cultured for the presence of P155 by streaking them directly onto plates of modified Drigalski medium (11, 18) containing 100 μg of nalidixic acid per ml. Blood cultures from representative live diarrheic calves or piglets were qualitatively cultured for strain B44 or P155. At necropsy, the heart, lungs, liver, and spleen of each calf or piglet were qualitatively cultured for strain B44 or P155. Contents of the jejunum, ileum, and colon of each calf were quantitatively cultured for strain B44; in piglets, contents of these organs were qualitatively cultured for strain P155. Jejunal, ileal, and colonic contents from several representative diarrheic piglets were quantitatively cultured for strain P155 by dilution in phosphate-saline and enumeration on modified Drigalski medium.

Medication. The monosodium salt of cephamycin C was used. All medication was given by gavage twice a day (b.i.d.) at 9:00 a.m. and 4:00 p.m. Immediately before dosing, the required amount of drug was dissolved in sterile water; calves were dosed with a 10-ml volume, and piglets were dosed with a 2-ml volume. In calves, the first regimen tested was 1,000 mg per calf b.i.d. for 6 days. The regimen was halved each time it was judged effective, resulting in the following numbers of calves for each regimen: 1,000 mg, 6; 500 mg, 5; 250 mg, 2; 125 mg, 4; 62.5 mg, 2; and 31.3 mg, 3. Finally, a regimen of 30 mg of cephamycin C per calf for 3 days was tested in seven calves.

Enterotoxin assays. Sterile broth filtrates of ETEC B44 or P155 and small intestinal and fecal samples obtained at necropsy from representative infected, nonmedicated diarrheic calves and piglets were individually assayed for ST activity in infant mice (10) and LT activity in adrenal (7) and Chinese hamster ovary cell cultures (9).

RESULTS

Calves. The disease syndrome which developed by 20 h PI was characterized by profuse watery diarrhea, severe dehydration, anorexia, prostration, and death generally at 2 days PI in nonmedicated calves. The jejunum, ileum, and colon of these calves each contained $>10^9$ CFU of strain B44 per g of contents. Samples from these three areas of the gut taken from representative calves produced gut weight/body weight ratios of >0.100 in infant mice, which indicated the presence of ST throughout the gut (10).

Cephamycin C at 31.3 to 1,000 mg per calf b.i.d. (0.75 to 24 mg/kg) for 6 days eliminated the diarrhea and other symptoms (Table 1). The efficacy at 31.3 mg per calf b.i.d. was comparable to all levels tested; therefore, the data for all calves medicated for 6 days were combined. Mortality was reduced from 90% in the infected, nonmedicated controls to 14% in the infected, cephamycin C-medicated calves. Medicated calves developed solid feces and shed significantly fewer CFU of B44 in their feces after the first day of treatment with cephamycin C. Diarrhea scores, weight loss, and mortality were comparable for infected, medicated calves and noninfected, nonmedicated control calves over the 10-day experimental period. Cephamycin C at 30 mg per calf (0.67 mg/kg) b.i.d. for 3 days instead of 6 days also eliminated the diarrhea and other symptoms (Table 2). Results were comparable to those presented in Table 1.

Piglets. The disease syndrome which developed by 6 h PI was characterized by profuse water diarrhea, severe dehydration, anorexia, prostration, and death generally at 1 day PI in nonmedicated piglets. Thus, the disease syndrome was very similar in calves and piglets. One noticeable difference was that the diarrhea appeared sooner after challenge in the piglets, and the nonmedicated piglets died sooner than did the nonmedicated calves. On necropsy, the entire gut of diarrheic piglets contained $>10^8$

TABLE 1. *Cephamycin C treatment (orally at 31.3 to 1,000 mg per calf [0.75 to 24 mg/kg] b.i.d. for 6 days) of E. coli enteritis in calves*[a]

Calves (no.)	Mortality[b] [deaths/total (%)]	Mean diarrhea score[c] at day PI:				Mean[d] B44 CFU/g of feces at day PI:				Mean weight loss [kg (%)]
		1	2	7	10	1	2	7	10	
Infected, nonmedicated (21)	19/21 (90)*	3.0	2.86*	—	—	2.6×10^9*	5.0×10^9*	—	—	6.4 (16)
Infected, medicated (22)	3/22 (14)**	3.0	0.77**	0.45	0.22	2.6×10^9*	6.0×10^6**	1.2×10^2	4.5×10^1	3.6 (8)*
Noninfected, nonmedicated (34)	2/34 (6)**	0.23	0.79**	0.41	0.21	—	—	—	—	2.3 (5)*

[a] Means for each variable with different numbers of asterisks are statistically significantly different ($P < 0.01$). Fisher's exact test was used to analyze the mortality data; diarrhea scores, bacterial counts, and weights were analyzed by analysis of variance and Duncan's new multiple range test. —, Not applicable.

[b] All deaths in infected, nonmedicated calves were due to induced enteritis; 15 deaths occurred on day 2 PI. In infected, medicated calves, two of three deaths were due to systemic infections, whereas in noninfected, nonmedicated calves, the two deaths were due to nonspecific enteritis.

[c] 3, Profuse, watery feces with little solid material; 2, watery feces with some solid material; 1, semisolid feces; 0, solid feces.

[d] Geometric mean.

TABLE 2. *Cephamycin C treatment (orally at 30 mg per calf [0.71 mg/kg] b.i.d. for 3 days) of E. coli enteritis in calves*[a]

Calves (no.)	Mortality[b] [deaths/total (%)]	Mean diarrhea score[c] at day PI:				Mean[d] B44 CFU/g of feces at day PI:				Mean weight loss [kg (%)]
		1	2	4	10	1	2	4	10	
Infected, nonmedicated (8)	5/8 (63)*	3.0	2.1*	—	—	2.8×10^9*	1.6×10^9*	—	—	5 (12)
Infected, medicated (7)	0/7 (0)**	3.0	0.71*	0.71	0.33	1.7×10^9*	6.9×10^6**	1.0×10^4	2.6×10^2	0.5 (2)*
Noninfected, nonmedicated (9)	2/9 (22)*,**	0.0	0.86*	1.17	0.29	—	—	—	—	2.7 (7)*

[a] Means for each variable with different numbers of asterisks are statistically significantly different ($P < 0.05$). —, Not applicable.

[b] All deaths in infected, nonmedicated calves occurred on day 2 PI and were due to induced enteritis; the two deaths in the noninfected, nonmedicated calves were due to nonspecific enteritis.

[c] 3, Profuse, watery feces with little solid material; 2, watery feces with some solid material; 1, semisolid feces; 0, solid feces.

[d] Geometric mean.

CFU of strain P155 per g of gut contents. Jejunal, ileal, and colonic contents of representative diarrheic piglets produced gut weight/body weight ratios of >0.100 in infant mice and caused rounding of adrenal cells and elongation of Chinese hamster ovary cells, which indicated the presence of ST and LT throughout the gut.

In caged piglets, cephamycin C given prophylactically (30 min before challenge) at 12.5 mg per piglet (10.4 mg/kg) b.i.d. for 4 days completely prevented both diarrhea and death in all nine piglets, whereas eight nonmedicated piglets had 100% diarrhea, and all died on day 1 PI. All nonmedicated piglets on necropsy had P155 throughout their gut, whereas in medicated piglets shedding of P155 was eliminated in eight of nine piglets by day 4 PI.

In a similar experiment, cephamycin C given therapeutically at 6 h PI reduced diarrhea and mortality (Fig. 1). Mortality was significantly ($P = 0.015$, Fisher's exact test) reduced from 79% (11 of 14) in the nonmedicated piglets to 23% (3 of 13) in the medicated piglets. Both medicated and nonmedicated piglets had severe diarrhea on day 1 PI. Of 14 nonmedicated piglets, 11 died on day 1 PI, and the surviving 3 piglets had diarrhea for the remainder of the experiment. In contrast, in the medicated piglets only two died on day 1 PI, and the diarrhea was significantly reduced ($P < 0.02$, McNemar's test [8]) by day 3 and eliminated ($P < 0.01$) by day 4 PI in the 11 surviving piglets (one piglet died with a diarrhea score of 2 on day 5 PI). Shedding of P155 in the feces was eliminated in 7 of 10 surviving piglets. In this experiment and the prophylactic experiment, seven of nine noninfected, nonmedicated control piglets survived and had solid feces to the end of the experiment. All eight noninfected, medicated control piglets survived, and seven of eight had solid feces to the end of the experiment.

In suckling piglets, cephamycin C given therapeutically at 6 h PI significantly reduced diarrhea and mortality. Table 3 shows the mortality data for this experiment. The reduction in mortality from 75 to 31% was significant ($P = 0.02$). In the suckling piglet experiments, the litter was considered the experimental unit. Thus, the mean mortality of 31% is skewed to the high side because of the 100% mortality in litter no. 5, which contained only 3 medicated piglets. If calculated on an individual piglet basis, the mortality would be 6 of 25 (24%), comparable to 23% in the caged piglet-therapy experiment.

The diarrhea data for this experiment are shown in Fig. 2. On day 1 PI there was significantly less ($P < 0.01$, paired t test on differences between litters) diarrhea in the medicated piglets versus the nonmedicated piglets. By day 2 PI the diarrhea was eliminated in the medicated piglets, and these piglets had solid feces throughout the remainder of the experiment. One noticeable difference between suckling piglets and the caged piglets was the faster response to treatment with cephamycin C as measured by the elimination of diarrhea. On day 1 PI the mean diarrhea score and percent diarrhea (number of piglets with a score of 2 or 3/total × 100) for the medicated caged piglets were 2.5 and 92% and not significantly different from the 2.6 and

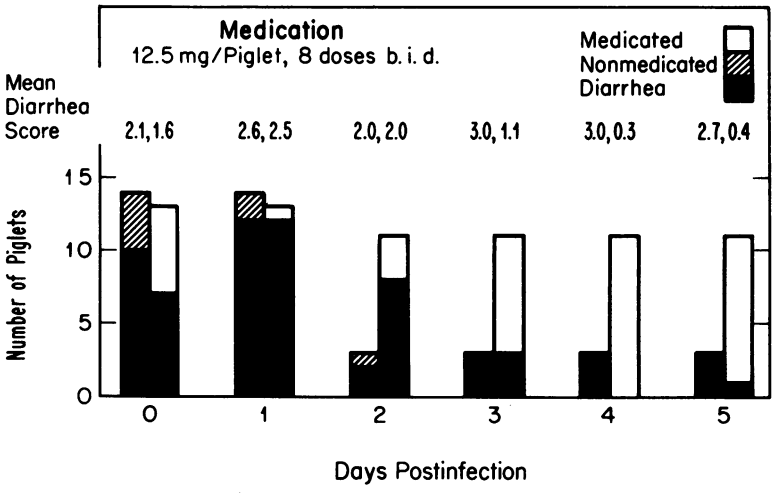

FIG. 1. *Cephamycin C treatment (orally at 12.5 mg per piglet [10.4 mg/kg] b.i.d. for 4 days) of E. coli enteritis in piglets. Diarrhea is defined as a score of 2 or 3. The day 0 PI mean diarrhea score is the score at 6 h PI (time of first medication). The last of eight doses of cephamycin C was given in the morning of day 4 PI. The number of piglets for each day PI equals surviving piglets plus piglets that died that day. One piglet in the infected, medicated group died on day 5 PI.*

TABLE 3. *Cephamycin C treatment (orally at 12.5 mg per piglet [10.4 mg/kg] b.i.d. for 3 days) of E. coli enteritis in suckling piglets*

Litter no.	Nonmedicated piglets				Medicated piglets			
	Alive	Dead	Total	% Mortality	Alive	Dead	Total	% Mortality
1 (gilt)	1	5	6	83	5	1	6	17
2 (sow)	3	3	6	50	4	1	5	20
3 (sow)	3	2	5	40	4	1	5	20
4 (sow)	0	6	6	100	6	0	6	0
5 (gilt)	0	4	4	100	0	3	3	100
Avg				75				31[a]

[a] Significant ($P = 0.02$) reduction in mortality (based on differences in mortality within litters; paired t test with double arc sine transformation).

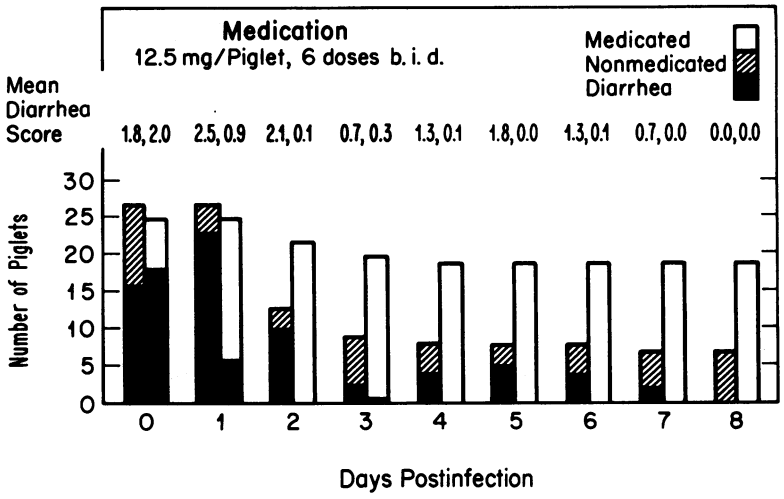

FIG. 2. *Diarrhea data for suckling piglets with E. coli enteritis treated with cephamycin C orally at 12.5 mg per piglet (10.4 mg/kg) b.i.d. for 3 days. Diarrhea is defined as a score of 2 or 3. The day 0 PI mean diarrhea score is the score at 6 h PI (time of first medication). The last of 6 doses of cephamycin C was given in the morning of day 3 PI. The number of piglets for each day PI equals surviving piglets plus piglets that died that day.*

86% for the nonmedicated piglets, whereas for the medicated suckling piglets the mean diarrhea score and percent diarrhea were 0.92 and 25%, each significantly lower than the 2.5 and 87% for the nonmedicated piglets. Diarrhea was eliminated by day 2 in the medicated, suckling piglets, whereas it took until day 4 PI to eliminate the diarrhea in the caged piglets.

DISCUSSION

This disease syndrome in calves was similar to that described by other investigators (1, 3, 14). In their reports and in this study calves became diarrheic 4 to 13 h PI and dehydrated rapidly; 80 to 90% died, generally at 24 to 72 h PI. The entire small and large bowel contained approximately 10^9 B44 CFU/g of bowel content. The B44 organisms were confined to the gut; septicemia did not occur until calves were moribund.

In piglets the disease syndrome was similar to that described by Nagy et al. (15), who used the same serotype of *E. coli* in suckling piglets. In both studies the onset of diarrhea was rapid, starting 4 to 6 h PI; the piglets dehydrated, and death generally occurred within 24 h PI. Of the challenged piglets, 90% became diarrheic and 74 to 80% died. As in calves, the entire small and large bowel contained approximately 10^9 P155 CFU/g of bowel content. The P155 organisms were confined to the gut; septicemia did not occur until the piglets were moribund. In the above-cited studies in both calves and piglets, vaccines were evaluated for their ability to prevent induced enteric colibacillosis. This study is the first to be reported in which an antibacterial agent was evaluated as a treatment for a highly lethal, induced enterotoxigenic colibacillosis in newborn, colostrum-fed calves or piglets.

Cephamycin C was highly effective in restor-

ing the calves and piglets to good health by eliminating diarrhea and reducing mortality. The antibacterial activity of cephamycin C is primarily bactericidal (13). In mice, cephamycin C given orally is essentially not absorbed (unpublished data). Absorption studies in calves or piglets have not been conducted, but the mice data would suggest the same for these animals. Thus, cephamycin C given orally remains in the gut, where it can best eliminate the enormous number of colonized enteropathogenic *E. coli*. Finally, in vitro studies (unpublished data) with multiply resistant enteropathogenic *E. coli* and *Salmonella* species have shown no resistance to this drug. The above, combined with its resistance to degradation by β-lactamases, make cephamycin C a potentially useful drug for treatment of colibacillosis in the livestock industry.

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