ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1988, p. 1769–1775
0066-4804/88/121769-07$02.00/0

Vol. 32, No. 12

Effects of Clindamycin and Metronidazole on the Intestinal Colonization and Translocation of Enterococci in Mice

CAROL L. WELLS,[1,2]* ROBERT P. JECHOREK,[2] MICHAEL A. MADDAUS,[1] AND RICHARD L. SIMMONS[1]†

Departments of Surgery[1] and Laboratory Medicine and Pathology,[2] University of Minnesota, Minneapolis, Minnesota 55455

Received 29 June 1988/Accepted 1 September 1988

The intestinal colonization and translocation of enterococci was studied in mice treated intramuscularly with metronidazole or clindamycin, with or without oral streptomycin. Treatment with metronidazole resulted in selective elimination of strictly anaerobic cecal bacteria, with a 100-fold increase in the numbers of aerobic and facultative gram-negative bacilli and a 10,000-fold increase in the numbers of aerobic and facultative gram-positive species. Clindamycin had a similar effect on the cecal flora except that the numbers of aerobic and facultative gram-positive bacteria decreased at least 10-fold. The predominating gram-positive species in the cecal flora of metronidazole-treated mice was an enterococcus, but this organism could not be recovered from the ceca of clindamycin-treated mice. Translocating bacteria (primarily gram-negative enteric bacteria) were recovered from the mesenteric lymph nodes of the majority of mice given metronidazole or clindamycin. Gram-positive bacteria were not recovered from the mesenteric lymph nodes of 20 clindamycin-treated mice, whereas 26% of 19 metronidazole-treated mice had translocating enterococci. With addition of streptomycin to the metronidazole and clindamycin regimens, mice treated with metronidazole-streptomycin became colonized predominantly with an enterococcus, and this was the only translocating species recovered from 13% of 23 mice; however, enterococci could not be detected in the ceca of clindamycin-streptomycin-treated mice, and *Bacillus* spp. were recovered from the mesenteric lymph nodes of 8% of 24 mice, reflecting the composition of the cecal flora. The apparent elimination of enterococci from the ceca of clindamycin and clindamycin-streptomycin-treated mice was inconsistent with the observation that the average (n = 6) peak levels of clindamycin in blood and ceca were 25 and 21 μg/ml, respectively, whereas the in vitro MIC was >128 μg/ml. However, this apparent in vivo activity of clindamycin against enterococci was not evident in mice given 10^9 oral enterococci; the concentrations of cecal enterococci in both clindamycin-streptomycin- and metronidazole-streptomycin-treated mice were 10^{10} to 10^{11} enterococci per g, with translocating enterococci recovered from approximately half of these antibiotic-treated mice. Thus, antibiotic therapy with metronidazole, clindamycin, metronidazole-streptomycin, and clindamycin-streptomycin resulted in a wide variation in the cecal population levels and translocation frequencies of enterococci. This variation appeared to be related to the discrepancy between the in vivo and in vitro activities of clindamycin against enterococci.

Enterococci are common pathogens in a variety of nosocomial infections, including urinary tract infections, wound infections, and bacteremias (1, 5, 8, 26). Although systemic enterococcal infections occur in nearly all types of hospitalized patients, they are most prevalent in surgical patients, who often have an undefined focus of infection (J. M. Hughes, D. R. Olson, T. G. Emori, W. R. Jarvis, D. H. Culver, and C. Thornsberry, Program Abstr. 25th Interscience Conference on Antimicrobial Agents and Chemotherapy, abstr. no. 1029, 1985). Because enterococcal infections frequently occur in patients who are receiving antibiotics directed primarily against enteric gram-negative bacilli, Dougherty et al. (9) studied the translocation of intestinal enterococci into extraintestinal tissues of streptomycin-treated mice and reported that streptomycin therapy promoted the intestinal colonization and translocation of orally inoculated enterococci. In this study (9), the invasive ability of enterococci confirmed an earlier report by van der Waaij et al. (23), who noted enterococcal translocation in mice given oral enterococci and parenteral streptomycin or ampicillin. Thus, there is evidence that enterococci can translocate out of the intestinal tracts of antibiotic-treated animals that have been orally inoculated with an exogenous strain of enterococcus.

We recently reported (25) that parenteral metronidazole therapy selectively eliminated all strictly anaerobic cecal bacteria in mice, resulting in intestinal overgrowth of aerobic and facultative gram-positive and gram-negative species, with concomitant translocation of these species into the mesenteric lymph nodes (MLN). These translocating bacteria were predominantly *Escherichia coli* and enterococci (25). To expand these studies, we next investigated the ability of clindamycin, another antibiotic active against anaerobes, to eliminate intestinal anaerobes and to promote the translocation of endogenous facultative bacteria. Preliminary experiments showed that, like metronidazole, clindamycin could eliminate all cecal anaerobic bacteria in mice. However, unlike metronidazole, clindamycin also had some effect on gram-positive species; translocating bacteria were exclusively facultative gram-negative bacilli, and no translocating enterococci were noted. The study reported here was designed with two specific aims: (i) to clarify the invasive potential of intestinal enterococci and (ii) to clarify the relative abilities of metronidazole and clindamycin to alter the intestinal flora and to modulate the intestinal colonization and translocation of enterococci.

* Corresponding author.
† Present address: Department of Surgery, University of Pittsburgh, Pittsburgh, PA 15261.

(A portion of this work has been previously presented [C. L. Wells, M. A. Maddaus, and R. L. Simmons, 27th ICAAC, abstr. no. 1339, 1987].)

MATERIALS AND METHODS

Antibiotic decontamination and oral inoculation of mice. Female 20- to 24-g Swiss Webster mice (Bio-Lab Corp., St. Paul, Minn.) were given various antimicrobial agents chosen for their particular effects on the intestinal flora. Separate groups of mice were given either clindamycin phosphate (The Upjohn Co., Kalamazoo, Mich.) or metronidazole (Searle Pharmaceuticals, Inc., Chicago, Ill.); additional groups of mice were given streptomycin sulfate (Sigma Chemical Co., St. Louis, Mo.) in combination with either clindamycin or metronidazole. Streptomycin was given in an attempt to selectively eliminate intestinal facultative gram-negative bacilli; 2 mg of this drug per ml was dissolved in the drinking water. Oral metronidazole has no noticeable killing effect on the total numbers of intestinal anaerobic bacteria in mice (3, 25). The reason for this is not known, but Hentges et al. (13) added metronidazole to the drinking water of mice and reported no detectable concentration of the drug in ceca. Preliminary experiments showed that oral clindamycin palmitate hydrochloride (Upjohn) also did not significantly alter the populations of intestinal bacteria in the mice. (However, oral uptake of the clindamycin-supplemented water might have been inadequate.) Mice were therefore given clindamycin or metronidazole intramuscularly twice a day in a dose of 8 or 4 mg/0.2 ml per mouse, respectively; preliminary experiments showed that these doses resulted in elimination of all detectable cecal anaerobic bacteria. Control mice received unsupplemented drinking water and twice-daily intramuscular injections of phosphate-buffered saline. Mice were sacrificed after 3 days of drug therapy, and the cecal and MLN bacteria were cultured as described below.

Cohort groups of mice treated with either clindamycin-streptomycin or metronidazole-streptomycin for 3 days were orally inoculated (with a feeding needle) with 0.1 ml of approximately 10^9 saline-washed enterococci (strain M20) from an overnight tryptic soy broth (Difco Laboratories, Detroit, Mich.) culture. Enterococcus M20 was isolated from the MLN of mice treated with metronidazole in our initial experiments. Mice were sacrificed 48 h after bacterial inoculation, and cecal and MLN bacteria were again cultured as described below. Antibiotic treatment was continued for the duration of the experiment.

Isolation and enumeration of viable cecal and translocating bacteria. Each mouse was killed by CO_2 asphyxiation, and the MLN was excised before excision of the cecum. Tissues were processed aseptically. The MLN were homogenized and quantitatively cultured as previously described (25). MLN homogenates were plated onto colistin-nalidixic acid agar (Difco) for enumeration of aerobic and facultative gram-positive bacteria and onto MacConkey agar (Difco) for enumeration of aerobic and facultative gram-negative bacilli; these agar plates were incubated aerobically at 35°C for 24 to 48 h. MLN bacteria were enumerated as the total number of viable bacteria per MLN. The MLN were cultured because it has been repeatedly shown that this is the most sensitive organ to culture in order to monitor the translocation of intestinal bacteria (25a). The data presented represent pooled data from similar replicate experiments performed on separate days, each experiment consisting of 10 to 12 mice per group.

Ceca were excised, weighed, and immediately transferred into an anaerobic chamber (Forma Scientific, Marietta, Ohio), where ceca were homogenized and serially diluted as previously described (25). These serial dilutions were plated onto the same media as were the MLN homogenates, with the addition of two plate media: (i) m enterococcus agar (Difco) supplemented with 10% bile salts for the enumeration of enterococci in the presence of other facultative gram-positive bacteria and (ii) Wilkins-Chalgren agar (Difco) supplemented with 100μg of gentamicin per ml (LyphoMed, Inc., Melrose Park, Ill.) for the enumeration of strictly anaerobic bacteria in the presence of large numbers of facultative bacteria. Preliminary experiments showed that similar numbers of anaerobic bacteria were recovered on Wilkins-Chalgren agar supplemented with gentamicin as were recovered on unsupplemented Wilkins-Chalgren agar. The m enterococcus agar plates were incubated aerobically for 48 h at 35°C, and the Wilkins-Chalgren agar plates were incubated anaerobically at 35°C for 72 to 96 h. Cecal bacteria were enumerated as the viable $\log_{10}$/g (wet weight) of cecum, and the limit of detection of the assay was 500 bacteria per cecum. The data presented represent pooled data from similar replicate experiments performed on separate days, each experiment consisting of four mice per group.

In one experiment (described in Results), peritoneal swabs were taken before excision of the MLN. These swabs were each placed in tryptic soy broth for 48 h at 35°C. Turbid broths were then subcultured to media described above for MLN homogenates, and all colony types were identified as described below.

Identification of bacteria. Bacteria were identified by standard techniques (10), with facultative gram-negative bacilli identified primarily by the API 20E system (Analytab Products, Plainview, N.Y.).

Histological sections of intestinal tissue. Intestinal tissues were excised from three mice in each experimental group. Duodenal tissue (5 cm proximal to the stomach), ileal tissue (5 cm proximal to the cecum), and cecal tissue (0.4 cm distal to the cecal tonsil) were placed in buffered Formalin until processed for histological sections and stained with hematoxylin and eosin stain and tissue Gram stain.

In vitro MIC of clindamycin for enterococci in mice. The in vitro MIC of clindamycin hydrochloride (susceptibility powder obtained from Upjohn) for enterococci was determined by a macrodilution assay (14), using Mueller-Hinton broth (Difco).

Quantitation of clindamycin in blood and cecal contents. Mice were injected intramuscularly with 8 mg of clindamycin phosphate (the dose used in all experiments reported here). Thirty minutes later, blood was collected from the tail vein for determination of clindamycin levels in a bioassay (2), using Mueller-Hinton agar (Difco) supplemented with 5% sheep blood and *Staphylococcus aureus* 6538P as the indicator organism. The mice were then sacrificed, and the ceca were removed, homogenized as a 1:3 dilution in phosphate-buffered saline, and filter sterilized. The resulting sterile filtrate was also tested in the bioassay, and the clindamycin concentration was determined after a correction for the dilution factor.

Bactericidal activity of cecal extracts. Five mice were injected intramuscularly with 8 mg of clindamycin phosphate and sacrificed 30 min later. The ceca were removed, pooled, homogenized as a 1:3 dilution in phosphate-buffered saline, and filter sterilized. This sterile filtrate was tested according to accepted methodology for a macrodilution serum bactericidal assay (19), with the cecal filtrate substituted for the serum in this method. Enterococcus M20 was the organism

TABLE 1. Characterization of cecal bacteria in eight mice treated for 3 days with either metronidazole or clindamycin administered parenterally

Antibiotic treatment	Log_{10} viable bacteria/g of cecum (avg ± SE)		
	Aerobic and facultative gram-negative bacilli	Aerobic and facultative gram-positive bacteria	Strict anaerobes
None	7.1 ± 0.4	6.0 ± 0.3	9.4 ± 0.3
Metronidazole	9.2 ± 0.7	10.1 ± 0.8[a]	ND[b]
Clindamycin	10.7 ± 0.2	4.6 ± 0.2[c]	ND

[a] Cecal bacteria were predominantly enterococci.
[b] ND, None detected.
[c] Cecal bacteria were predominantly lactobacilli and *Bacillus* spp.; no enterococci could be detected. Numbers of viable bacteria were significantly lower than in control and metronidazole-treated mice ($P < 0.01$ and $P < 0.001$, respectively).

tested. The assay was repeated by using another group of five mice.

RESULTS

Effects of clindamycin, metronidazole, clindamycin-streptomycin, and metronidazole-streptomycin on the gross and microscopic intestinal anatomy. In all treatment groups, light microscopy of duodenal, ileal, or cecal tissue showed no remarkable pathological findings. However, in some of the mice heavily colonized with enterococci (see below), clusters of gram-positive cocci were adherent to the intestinal mucosa; the interactions of enterococci with the intestinal epithelium will be described in a subsequent manuscript (C. L. Wells et al., manuscript in preparation). All mice treated with metronidazole, clindamycin, metronidazole-streptomycin, or clindamycin-streptomycin had relatively fluid cecal material. This diarrhea appeared most severe in the metronidazole-streptomycin-treated group, where anal soiling was evident. Approximately 20 to 40% of the clindamycin-streptomycin-treated mice (before and after oral administration of enterococci) had grossly enlarged stomachs (approximately three to four times normal size). Abdominal adhesions were noted in occasional mice (approximately 20%) that had been given metronidazole-streptomycin without oral enterococci. The abdominal cavities of mice given metronidazole-streptomycin and oral enterococci contained noticeable amounts of exudative fluid, intestinal adhesions were present, and the MLN were difficult to excise. Because this latter group of mice appeared to have peritonitis, peritoneal swabs were cultured in the second of the two experiments in which mice received oral enterococci and either metronidazole-streptomycin or clindamycin-streptomycin. In this experiment, all peritoneal swabs from control and clindamycin-streptomycin-treated mice were sterile, whereas 80% of the peritoneal swabs from the metronidazole-streptomycin-treated mice yielded a pure culture of enterococci, confirming the gross observation of peritonitis.

Cecal and MLN bacteria of mice treated with metronidazole or clindamycin. All detectable anaerobic bacteria were eliminated from the ceca of mice injected with either metronidazole or clindamycin, and the numbers of indigenous cecal enteric gram-negative bacilli increased 100- to 1,000-fold, respectively (Table 1). Cecal aerobic and facultative gram-positive bacteria increased 10,000-fold in metronidazole-treated mice, with an enterococcus as the predominant species; in contrast, cecal aerobic and facultative gram-positive bacteria decreased at least 10-fold in clindamycin-treated mice, and enterococci could not be detected (Table 1).

Occasional untreated mice (20%) had viable bacteria recovered from the MLN, (Table 2) a typical observation for untreated mice (reviewed in 25a). In contrast, the majority of mice treated with either metronidazole or clindamycin had viable bacteria in the MLN. In metronidazole-treated mice, the majority of these translocating bacteria were gram-negative coliforms, with *E. coli* as the predominant isolate; however, 5 of 19 (26%) of these mice had translocating gram-positive bacteria, and enterococci were isolated from the MLN of each of these latter 5 mice (Table 2). In clindamycin-treated mice, the MLN bacteria were exclusively gram-negative coliforms, and again *E. coli* was the predominant isolate; no enterococci or any other gram-positive bacteria were recovered from the MLN of clindamycin-treated mice (Table 2).

Cecal and MLN bacteria of mice treated with metronidazole-streptomycin or clindamycin-streptomycin. The results of the experiments described above indicated that metronidazole, but not clindamycin, could be used to promote the intestinal colonization and translocation of enterococci. Streptomycin was then added to each of these drug regimens in an attempt to eliminate facultative gram-negative bacilli and to further enhance the growth of intestinal enterococci.

TABLE 2. Effects of metronidazole and clindamycin on the translocation of intestinal bacteria into the MLN of mice[a]

Antibiotic treatment[a]	No. of mice with bacteria in MLN/total no. of mice	No. of mice with enterococci in MLN/total no. of mice	No. and identity of translocating species in individual mice
None	2/20	0/20	160 alpha-hemolytic streptococci, 20 *Escherichia coli*
Metronidazole	15/19[b,d]	5/19[c]	10 *E. coli* (2 mice), 30 *E. coli* (2 mice), 10 *Enterobacter cloacae*, 20 *E. coli* plus 90 *E. cloacae*, 40 *E. coli* type A–D, 20 *Proteus mirabilis* (2 mice), 60 *E. coli* plus 30 *P. mirabilis*, 120 *E. coli* plus 230 *P. mirabilis* plus 10 enterococci, 730 *E. coli* plus 160 *P. mirabilis* plus 3,000 enterococci plus 2,000 alpha-hemolytic streptococci, 10 enterococci, 20 enterococci, 30 enterococci
Clindamycin	14/20[d]	0/20	10 *E. coli* (7 mice), 20 *E. coli* (2 mice), 30 *E. coli*, 90 *E. coli*, 10 *E. coli* plus 10 *E. cloacae*, 10 *Klebsiella pneumoniae*, 30 *E. coli* plus 40 *Citrobacter freundii*

[a] Pooled data from two experiments with 9 to 10 mice per group in each experiment.
[b] One mouse died.
[c] Significantly higher than in untreated and clindamycin-treated mice ($P < 0.05$).
[d] Significantly higher than in untreated mice ($P < 0.01$).

TABLE 3. Characterization of endogenous cecal bacteria in eight mice treated for 3 days with metronidazole-streptomycin or clindamycin-streptomycin

Antibiotic treatment	Log_{10} viable bacteria/g of cecum (avg ± SE)			
	Aerobic and facultative gram-negative bacilli[a]	Aerobic and facultative gram-positive bacteria		Strict anaerobes
		Total	Enterococci	
None	6.0 ± 0.5	8.9 ± 0.2	4.4 ± 1.3	9.5 ± 0.3
Metronidazole-streptomycin	ND[b]	9.4 ± 0.7[c]	9.5 ± 0.7	ND
Clindamycin-streptomycin	ND	6.2 ± 0.2[d]	ND[e]	ND

[a] No streptomycin-resistant gram-negative bacilli were detected in any mouse in any treatment group.
[b] ND, None detected.
[c] Cecal bacteria were predominantly enterococci, but *Bacillus* spp. and lactobacilli were detected in approximately 1,000-fold-lower numbers.
[d] Cecal bacteria were predominantly *Bacillus* spp., and no enterococci could be detected.
[e] Significantly lower than in the control or metronidazole-streptomycin group ($P < 0.001$).

Characterization of the cecal flora of mice treated with either metronidazole-streptomycin or clindamycin-streptomycin is presented in Table 3. (Fortunately, no streptomycin-resistant gram-negative bacilli were cultured from any mouse, in any treatment group, for the duration of these experiments.) Control mice had relatively low numbers of cecal enterococci (Table 3); in this group there was noticeable animal-to-animal variation, and although indigenous enterococci were recovered from each mouse assayed, the quantitation ranged from 10^3 to 10^7/g of cecum. Predictably, metronidazole-streptomycin therapy eliminated all cecal bacteria except aerobic and facultative gram-positive bacteria, with high numbers of enterococci as the predominant species (Table 3). Clindamycin-streptomycin also eliminated all cecal bacteria except aerobic and facultative gram-positive bacteria; however, in this case the remaining gram-positive bacteria were predominantly *Bacillus* spp. (with lower numbers of lactobacilli), and no enterococci were detected (Table 3).

Metronidazole-streptomycin therapy was associated with intestinal overgrowth of indigenous enterococci (Table 3), and this was the only organism recovered from the MLN of occasional mice (13%, 3 of 23) in this treatment group (Table 4). In contrast, the predominant members of the gram-positive cecal flora in clindamycin-streptomycin-treated mice (Table 3) were *Bacillus* spp., and these were the only organisms recovered from the MLN of occasional mice (8%, 2 of 24) in this treatment group (Table 4).

Susceptibility to clindamycin of enterococci in mice. Because clindamycin appeared to prevent the intestinal colonization and translocation of enterococci in both clindamycin- and clindamycin-streptomycin-treated mice (Tables 1 through 4), the blood and cecal levels of clindamycin, as well as the in vitro MIC of clindamycin for enterococci, were determined. The in vitro MICs for six enterococcal isolates from six mice treated with metronidazole-streptomycin (four MLN and two cecal isolates) were each >128 μg/ml. Thirty minutes after intramuscular injection of 8 mg of clindamycin (the dose of clindamycin used in these studies) into each of six mice, the clindamycin concentrations were 25 ± 3 μg/ml (average ± standard error) in whole blood and 21 ± 4 μg/ml (average ± standard error) in cecal homogenates. In addition, cecal extracts from mice injected with clindamycin were substituted for serum in a classical serum bactericidal assay, and there was no detectable in vitro antienterococcal activity in these cecal extracts.

Cecal and MLN bacteria in mice treated with metronidazole-streptomycin or clindamycin-streptomycin and orally inoculated with enterococci. In a further attempt to increase the colonization and translocation of intestinal enterococci, each mouse treated with either metronidazole-streptomycin or clindamycin-streptomycin was orally inoculated with 10^9 enterococci and sacrificed 2 days later. At this time, control mice given oral enterococci without antimicrobial therapy had relatively low numbers of cecal enterococci compared with their antibiotic-treated counterparts (Table 5). In fact, the numbers of cecal enterococci in mice given oral enterococci but no antimicrobial therapy were not significantly different from those of untreated mice that had not received 10^9 oral enterococci (Table 3). After receiving oral enterococci, both metronidazole-streptomycin- and clindamycin-streptomycin-treated mice had high numbers of cecal enterococci, i.e., approximately 10^{11}/g of cecum (Table 5).

Translocating bacteria recovered from the MLN of antibiotic-treated mice orally inoculated with enterococci are presented in Table 6. Control mice given no antibiotics had similarly low translocation incidences both before (1 of 24, Table 4) and after (1 of 23, Table 6) receiving oral enterococci. After administration of oral enterococci, the numbers of cecal enterococci (Table 5), as well as the incidences of enterococcal translocation (Table 6), were similar in metronidazole-streptomycin- and clindamycin-streptomycin-treated mice. The incidences of enterococcal translocation in both metronidazole-streptomycin- and clindamycin-streptomycin-treated mice were significantly greater than in the cohort groups not given oral enterococci (comparison of results in Tables 4 and 6).

TABLE 4. Effects of clindamycin-streptomycin and metronidazole-streptomycin on the translocation of endogenous intestinal bacteria into the MLN of mice[a]

Antibiotic treatment	No. of mice with bacteria in MLN/total no. of mice	No. of mice with enterococci in MLN/total no. of mice	No. and identity of translocating species in individual mice
None	5/24	1/24	10 *E. coli*, 20 *E. coli*, 70 *E. coli*, 70 lactobacilli, 60 enterococci plus 490 alpha-hemolytic streptococci
Metronidazole-streptomycin	3/23[b]	3/23[c]	10, 40, 380 (all enterococci)
Clindamycin-streptomycin	2/24	0/24	10 *Bacillus* spp., 20 *Bacillus* spp.

[a] Pooled data from two experiments with 12 mice per experiment.
[b] One mouse died.
[c] Significantly higher than in the clindamycin-streptomycin group ($P < 0.05$).

TABLE 5. Characterization of cecal bacteria in eight mice treated for 3 days with metronidazole-streptomycin or clindamycin-streptomycin and subsequently orally inoculated with 10^9 enterococci

Antibiotic treatment	Log_{10} viable bacteria/g of cecum (avg ± SE)			
	Aerobic and facultative gram-negative bacilli[a]	Aerobic and facultative gram-positive bacteria		Strict anaerobes
		Total	Enterococci	
None	5.5 ± 0.5	8.2 ± 0.1	5.3 ± 0.5	9.6 ± 0.2
Metronidazole-streptomycin	ND[b]	10.9 ± 0.2[c]	10.9 ± 0.2	ND
Clindamycin-streptomycin	ND	10.6 ± 0.4[c]	10.6 ± 0.5	ND

[a] No streptomycin-resistant gram-negative bacilli were detected in any mouse in any treatment group.
[b] ND, None detected.
[c] Cecal bacteria were predominantly enterococci, but *Bacillus* spp. and lactobacilli were detected in 1,000- to 10,000-fold-lower numbers.

DISCUSSION

The experiments reported here were designed to investigate the relative effects of metronidazole, clindamycin, metronidazole-clindamycin, and clindamycin-streptomycin on the composition of the intestinal flora and on the translocation of intestinal bacteria. It was noted that these antimicrobial agents could be used to study the relationship between the concentration of intestinal enterococci and the frequency of enterococcal translocation into MLN. An unexpected observation was an apparent discrepancy between the in vitro and in vivo susceptibilities of indigenous enterococci to clindamycin.

Three days of parenteral therapy with either clindamycin or metronidazole resulted in the elimination of all detectable cecal anaerobic bacteria in mice, facilitating the overgrowth and translocation of endogenous aerobic and facultative species (Tables 1 and 2). In agreement with previous findings (25), metronidazole facilitated the cecal overgrowth of both gram-positive and gram-negative bacteria. In contrast, clindamycin facilitated the cecal overgrowth of gram-negative enteric bacteria while inhibiting the colonization of gram-positive bacteria (Table 1). An enterococcus was the predominant gram-positive species in the ceca of metronidazole-treated mice, but this organism was not detected in the cecal flora of clindamycin-treated mice (Table 1). The overall frequencies of bacterial translocation were similar in metronidazole- and clindamycin-treated mice and significantly higher than in control mice (Table 2). The bacterial species recovered from the MLN of clindamycin-treated mice were exclusively members of the family *Enterobacteriaceae*, with *E. coli* the predominant isolate. However, there were a variety of translocating species recovered from the MLN of metronidazole-treated mice; in this group *E. coli* and enterococci were the predominant isolates, with other members of the family *Enterobacteriaceae* and streptococci also represented.

Of interest was the observation that enterococci translocated to the MLN of only 26% (5 of 19) of metronidazole-treated mice (Table 2), i.e., mice that had 10^{10} enterococci per g of cecum (Table 1). This translocation incidence seemed relatively low compared with the 90 to 100% incidence of *E. coli* translocation in antibiotic-treated mice similarly colonized with 10^{10} to 10^{11} *E. coli* per g of cecum (4, 21, 25). To better compare the invasive abilities of enterococci and *E. coli*, streptomycin was added to the metronidazole and clindamycin regimens in order to eliminate facultative gram-negative bacilli and possibly facilitate the colonization of endogenous enterococci. After metronidazole-streptomycin treatment, however, the cecal flora consisted exclusively of aerobic and facultative gram-positive bacteria (Table 3), and the concentration of enterococci was similar to that of mice treated with metronidazole alone (Table 1); likewise, the incidences of enterococcal translocation were similar in the two groups of mice (Tables 2 and 4). In contrast, the cecal flora of clindamycin-streptomycin-treated mice consisted exclusively of aerobic and facultative gram-positive bacteria and enterococci were not detected, despite the presence of indigenous enterococci in the ceca of untreated animals (Table 3). Thus, clindamycin appeared to eliminate intestinal enterococci, a curious finding because enterococci are typically resistant to clindamycin in vitro (6). Predictably, no translocating enterococci were recovered from the MLN of clindamycin-streptomycin-treated mice, and an occasional mouse in this group had translocating *Bacillus* spp. (Table 4), reflecting the composition of the cecal flora (Table 3).

In a recent study, Dougherty et al. (9) administered exogenous enterococci to streptomycin-treated mice and noted that enterococcal translocation could be prevented by LY146032, an experimental drug with in vitro activity against enterococci. The results of our study differ in that an antibiotic with no in vitro activity against enterococci,

TABLE 6. Effects of clindamycin-streptomycin and metronidazole-streptomycin on the translocation of intestinal bacteria into the MLN of mice after oral inoculation with 10^9 enterococci[a]

Antibiotic treatment	No. of mice with bacteria in MLN/total no. of mice	No. of mice with enterococci in MLN/total no. of mice	No. and identity of translocating species in individual mice
None	3/23	1/23	10 *E. coli* (2 mice), 10 enterococci
Metronidazole-streptomycin	13/23	11/23[b]	10 lactobacilli, 110 lactobacilli, 10, 10, 10, 20, 20, 50, 70, 90, 140, 180, 220 (all enterococci)
Clindamycin-streptomycin	9/23	9/23[b]	10, 10, 10, 10, 10, 20, 40, 150, 560 (all enterococci)

[a] Pooled data from two experiments with 11 to 12 mice per experiment.
[b] Significantly higher than in the cohort group (Table 4) before administration of oral enterococci ($P < 0.01$).

namely, clindamycin, appeared to inhibit the intestinal replication and translocation of endogenous (not exogenous) enterococci. To clarify the susceptibility to clindamycin of enterococci in mice, results for the in vitro susceptibility of enterococci were compared with the levels of clindamycin in blood and ceca that were pertinent to this study. Six different enterococcal isolates from mice were resistant to clindamycin at an MIC of >128 μg/ml, whereas the peak levels of clindamycin in blood and ceca were consistently below 30 μg/ml. Thus, there was a discrepancy between the in vitro and in vivo susceptibilities of enterococci to clindamycin. There are myriad explanations for this observation, all dependent on the inherent differences between in vitro and in vivo susceptibility assays, e.g., the size of the bacterial inoculum, the availability of nutrients, the presence of toxic compounds, the redox potential in the infected site, and the presence of the immune system. We have recently presented evidence that mononuclear phagocytes can transport intestinal particles to the draining MLN (24, 25a). In addition, there is evidence suggesting that clindamycin can augment phagocytic elimination of certain bacterial species, including streptococci (11, 17, 18). If bacterial translocation is mediated by phagocytic cells, it is therefore reasonable to speculate that clindamycin could have interfered with translocation by augmenting the in vivo phagocytic uptake and intracellular killing, or both, of translocating enterococci.

There is yet another explanation for the discrepancy between the in vitro and in vivo activities of clindamycin against intestinal enterococci. Although bile represents a minor route of excretion of parenteral clindamycin, a metabolite (such as *N*-D-methyl clindamycin) that had in vivo activity against enterococci might have been excreted into the bowel (6, 16). Therefore, by using a modified serum bactericidal assay (cecal extract substituted for serum), cecal extracts from clindamycin-treated mice were tested for antienterococcal activity. However, no antienterococcal activity was detected in these cecal extracts, which indicated that the apparent in vivo activity of clindamycin against enterococci was not due to an active clindamycin metabolite that escaped detection by bioassay.

The susceptibility of enterococci to metronidazole was not studied because it is widely accepted that metronidazole has no activity against enterococci and, in this study, metronidazole therapy appeared to promote the intestinal colonization of endogenous enterococci. In a somewhat analogous experimental situation, Onderdonk and Cisneros (20) reported the relative effects of metronidazole and clindamycin on the development of experimental intraabdominal abscesses initiated with *Bacteroides fragilis* and *Streptococcus intermedius*; all metronidazole-treated animals developed abscesses containing pure cultures of *S. intermedius*, whereas no bacteria were cultured from abscesses in the clindamycin treatment group. It is tempting to speculate that the lack of activity of metronidazole against enterococci (and other streptococci) may be related to the increasing number of clinical reports of complicating streptococcal infections in metronidazole-treated patients (12, 15, 22).

To further challenge the in vivo susceptibility of enterococci to clindamycin and further increase the concentration of cecal enterococci, mice treated with metronidazole-streptomycin or clindamycin-streptomycin were orally inoculated with 10^9 enterococci. Forty-eight hours later, mice given no antibiotics had eliminated at least 99.99% of this inoculum, whereas the resulting cecal concentrations in both groups of antibiotic-treated mice were at similarly high levels of 10^{11}/g (Table 5). Similar translocation incidences of 49% (11 of 23) and 39% (9 of 23) were noted in these metronidazole-streptomycin- and clindamycin-streptomycin-treated mice, respectively (Table 6). Thus, an oral inoculation of a relatively large number of enterococci appeared to overcome the in vivo ability of clindamycin to prevent the intestinal colonization and translocation of this organism. (Unfortunately, smaller inocula of enterococci, i.e., inocula that might be more relevant to the clinical situation, were not tested.) It should be noted that in these mice heavily colonized with enterococci, the translocation incidences of approximately 40 to 50% noted above were somewhat less than the 90 to 100% incidences reported for animals similarly colonized with high concentrations of *E. coli* (4, 21, 25). Steffen and Berg (21) studied gnotobiotic mice disassociated with *E. coli* and *Streptococcus* (*Enterococcus*) *faecalis* and similarly concluded that enterococci failed to translocate whereas *E. coli* translocated in all disassociated mice; it should be noted that in this study, *E. coli* colonized the ceca at approximately 10^{10}/g whereas the concentration of enterococci was 100-fold less. In our study, mice given antibiotics plus oral enterococci had extremely high cecal concentrations of enterococci (nearly 10^{11}/g [Table 5]), yet enterococci translocated to the MLN of less than 50% of the mice (Table 6). Therefore, the invasive ability of enterococci appeared to be less than that reported for *E. coli* studied under similar conditions of intestinal overgrowth. A clarification of the invasive properties of *E. coli* and enterococci may be helpful in clarifying the bacterial properties favoring translocation to the MLN.

The observations and conclusions derived from this study can be summarized as follows: (i) the intestinal overgrowth and translocation of endogenous enterococci was facilitated with both metronidazole and metronidazole-streptomycin but not with clindamycin or clindamycin-streptomycin; (ii) the incidence of enterococcal translocation appeared to be positively correlated with the concentration of cecal enterococci, but even if enterococci were present at high cecal concentrations of 10^{11}/g, the incidence of enterococcal translocation was somewhat less than that reported for *E. coli*; (iii) both metronidazole-streptomycin and clindamycin-streptomycin promoted the intestinal replication and translocation of enterococci administered as a relatively large oral inoculum; (iv) there was a discrepancy between the in vitro and in vivo susceptibilities of endogenous enterococci to clindamycin, but this discrepancy was negated after enterococci were administered as a relatively large oral inoculum; and (v) animal models of bacterial translocation may be relevant and sensitive models in which to study the in vivo effects of antimicrobial agents.

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service grants AI23484 and AI14032 from the National Institutes of Health and by a grant from The Upjohn Co.

LITERATURE CITED

1. **Ahrenholtz, D. H., and R. L. Simmons.** 1988. Peritonitis and other intraabdominal infections, p. 605–646. *In* R. J. Howard and R. L. Simmons (ed.), Surgical infectious diseases, 2nd ed. Appleton & Lange, East Norwalk, Conn.
2. **Anhalt, J. P.** 1985. Assays for antimicrobial agents in body fluids, p. 1009–1014. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
3. **Berg, R. D.** 1981. Promotion of the translocation of enteric bacteria from the gastrointestinal tracts of mice by oral treat-

ment with penicillin, clindamycin, or metronidazole. Infect. Immun. 33:854–861.
4. **Berg, R. D., and A. V. Garlington.** 1979. Translocation of certain indigenous bacteria from the gastrointestinal tract to the mesenteric lymph nodes and other organs in a gnotobiotic mouse model. Infect. Immun. **23**:403–411.
5. **Chandrasekar, P. H., B. R. Smith, J. L. LeFrock, and B. Carr.** 1984. Enterococcal superinfection and colonization with aztreonam therapy. Antimicrob. Agents Chemother. **26**:280–282.
6. **DeHaan, R. M., C. M. Metzler, D. Schnellenberg, and W. D. Vandenbosch.** 1973. Pharmacokinetic studies of clindamycin phosphate. J. Clin. Pharmacol. **13**:190–209.
7. **Dhawan, V. K., and H. Thadepalli.** 1982. Clindamycin: a review of fifteen years of experience. Rev. Infect. Dis. **4**:1133–1153.
8. **Dougherty, S. H., A. B. Flohr, and R. L. Simmons.** 1983. "Breakthrough" enterococcal septicemia in surgical patients: 19 cases and review of the literature. Arch. Surg. **118**:232–237.
9. **Dougherty, S. H., D. J. Hentges, S. W. Casey, and W. A. Thal.** 1988. Impact of LY146032 on *Streptococcus* (*Enterococcus*) *faecalis* translocation in mice. Antimicrob. Agents Chemother. **32**:337–340.
10. **Finegold, S. M., and E. J. Baron.** 1986. Bailey and Scott's diagnostic microbiology. The C. V. Mosby Co., St. Louis.
11. **Gemmell, C. G., P. K. Peterson, D. Schmeling, Y. Kim, J. Mathews, L. Wannamaker, and P. J. Quie.** 1981. Potentiation of opsonization and phagocytosis of *Streptococcus pyogenes* following growth in the presence of clindamycin. J. Clin. Invest. **67**:1249–1256.
12. **Goepel, J. R., D. G. Richards, D. M. Harris, and L. Henry.** 1980. Fulminate streptococcus pyogenes infection. Br. Med. J. **281**: 1412.
13. **Hentges, D. J., A. J. Stein, S. W. Casey, and J. U. Que.** 1985. Protective role of intestinal flora against infection with *Pseudomonas aeruginosa* in mice: influence of antibiotics on colonization resistance. Infect. Immun. **49**:654–663.
14. **Jones, R. N., A. L. Barry, T. L. Gavan, and J. A. Washington II.** 1985. Susceptibility tests: microdilution and macrodilution broth procedure, p. 972–977. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
15. **Madden, N. P., and C. A. Hart.** 1985. *Streptococcus milleri* in appendicitis in children. J. Pediatr. Surg. **20**:6–7.
16. **McCall, C. E., N. H. Steigbigel, and M. Finland.** 1967. Lincomycin: activity in vitro and absorption and excretion in normal young men. Am. J. Med. Sci. **254**:144–155.
17. **Milatovic, D.** 1983. Antibiotics and phagocytosis. Eur. J. Clin. Microbiol. **2**:414–425.
18. **Milatovic, D., I. Braveny, and J. Verhoef.** 1983. Clindamycin enhances opsonization of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **24**:413–417.
19. **National Committee for Clinical Laboratory Standards.** 1987. Methodology for the serum bactericidal test, vol. 7, no. 1. Document M21-P. National Committee for Clinical Laboratory Standards, Villanova, Pa.
20. **Onderdonk, A. B., and R. Cisneros.** 1985. Comparison of clindamycin and metronidazole for the treatment of experimental intraabdominal sepsis produced by *Bacteroides fragilis* and *Streptococcus intermedius*. Curr. Ther. Res. **38**:893–898.
21. **Steffen, E. K., and R. D. Berg.** 1983. Relationship between cecal population levels of indigenous bacteria and translocation to the mesenteric lymph nodes. Infect. Immun. **39**:1252–1259.
22. **Tresadern, J. C., R. J. Farrand, and M. H. Irving.** 1983. *Streptococcus milleri* and surgical sepsis. Ann. R. Coll. Surg. Engl. **65**:78–79.
23. **van der Waaij, D., J. M. Berghuis, and J. E. C. Lekkerkerk.** 1972. Colonization resistance of the digestive tract of mice during systemic antibiotic treatment. J. Hyg. **70**:605–609.
24. **Wells, C. L., M. A. Maddaus, S. L. Erlandsen, and R. L. Simmons.** 1988. Evidence for the phagocytic transport of intestinal particles in dogs and rats. Infect. Immun. **56**:278–282.
25. **Wells, C. L., M. A. Maddaus, C. M. Reynolds, R. P. Jechorek, and R. L. Simmons.** 1987. Role of anaerobic flora in the translocation of aerobic and facultatively anaerobic intestinal bacteria. Infect. Immun. **55**:2689–2694.
25a. **Wells, C. L., M. A. Maddaus, and R. L. Simmons.** 1988. Proposed mechanisms for the translocation of intestinal bacteria. Rev. Infect. Dis. **10**:958–979.
26. **Zervos, M. J., C. A. Kauffman, P. M. Therasse, A. G. Bergman, T. S. Mikesell, and D. R. Schaberg.** 1987. Nosocomial infection by gentamicin-resistant *Streptococcus faecalis*. An epidemiologic study. Ann. Intern. Med. **106**:687–691.