ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1991, p. 976–982
0066-4804/91/050976-07$02.00/0

Vol. 35, No. 5

Modulation of the Intestinal Flora of Mice by Parenteral Treatment with Broad-Spectrum Cephalosporins

M. L. VAN OGTROP,[1,2] H. F. L. GUIOT,[1,2] H. MATTIE,[1]* AND R. VAN FURTH[1]

Department of Infectious Diseases, University Hospital,[1] and J. A. Cohen Institute for Radiopathology and Radiation Protection,[2] Leiden, The Netherlands

Received 1 August 1990/Accepted 27 December 1990

A study was performed to determine the effect of parenteral treatment with four broad-spectrum cephalosporins (cefoperazone, ceftriaxone, ceftazidime, and cefepime) on the number of aerobic gram-negative rods and on the outgrowth of *Candida albicans* and a multiresistant strain of *Citrobacter freundii* in the feces of mice. The estimated fractions of a parenteral dose that were excreted into the gastrointestinal tract were 0.37 for cefoperazone, 0.11 for ceftriaxone, 0.03 for ceftazidime, and 0.002 for cefepime. All four cephalosporins significantly decreased the number of aerobic gram-negative rods in the feces, and virtually all gram-negative rods were eliminated at high doses of cefoperazone, ceftazidime, and ceftriaxone. Furthermore, at high doses these three compounds led to a significant increase of the outgrowth of resistant *Citrobacter freundii*. The outgrowth of *Candida albicans* was increased at high doses of cefoperazone and ceftriaxone, whereas ceftazidime and cefepime did not have this effect. The most profound changes in the gastrointestinal ecology were observed during treatment with high doses of cefoperazone. The results suggest that the colonization resistance of the gastrointestinal tract can be substantially decreased by parenteral treatment with cefoperazone and, to a lesser extent, with ceftriaxone and ceftazidime.

Broad-spectrum cephalosporins are frequently used for empirical treatment and for the treatment of gram-negative infections in granulocytopenic patients (15). These compounds possess a powerful in vitro activity against gram-negative bacteria, including *Pseudomonas aeruginosa*. Most gram-positive bacteria are also considered susceptible.

Broad-spectrum cephalosporins may also disturb the anaerobic and aerobic intestinal microflora (1, 2, 5), which can lead to a decrease of colonization resistance to opportunistic pathogens. This may lead subsequently to overgrowth by fungi or multiresistant bacteria, which—especially in granulocytopenic patients—increases the risk of acquiring a nosocomial infection.

The degree of disturbance of the intestinal microflora due to the parenteral administration of antibacterial agents is dependent on the intrinsic activity of the antibacterial agent against the indigenous bacteria of the gastrointestinal tract as well as on the pharmacokinetic characteristics of the antibacterial agent determining the concentration of the agent in the gastrointestinal tract.

In the present study, the effect of parenteral administration of four broad-spectrum cephalosporins (cefoperazone, ceftriaxone, ceftazidime, and cefepime) on the aerobic gram-negative fecal flora and the colonization resistance of the intestinal tract was determined. These four drugs were chosen for several reasons. Cefoperazone was included because it has broad-spectrum activity against intestinal anaerobic bacteria and is excreted predominantly in the bile (6); ceftriaxone was included because it is also excreted into the bile (23), but it lacks broad antianaerobe activity. Ceftazidime was studied because it lacks broad antianaerobe activity and, unlike cefoperazone and ceftriaxone, is mainly excreted by the kidney (22); and cefepime was studied because, like ceftazidime, it is mainly excreted by the kidney (9), but its antibacterial spectrum includes gram-positive cocci such as *Staphylococcus aureus* (13, 14, 25). Comparison of the effects of these four antibacterial agents was expected to provide information about the relationship among the effect of broad-spectrum cephalosporins on gastrointestinal flora, their in vitro antibacterial spectrum, and their pharmacokinetic characteristics.

MATERIALS AND METHODS

Antibiotics. Cefoperazone (sodium salt, activity 96.4%) was kindly donated by Pfizer Pharmaceuticals, Rotterdam, The Netherlands; ceftazidime (activity 84.2%) was donated by Glaxo, Nieuwegein, The Netherlands; ceftriaxone (activity 83.95%) was donated by Hoffmann-La Roche, Basel, Switzerland; and cefepime (activity 82.6%) was donated by Bristol Myers, Brussels, Belgium. Just before use, solutions of the drugs were prepared freshly in phosphate-buffered saline (PBS), according to the manufacturer's instructions.

Animals. Female specific-pathogen-free Swiss mice (Broekman Institutes, Someren, The Netherlands), aged 5 to 6 weeks, were used throughout the study. The animals were housed in groups of four to five in polycarbonate cages on sterile sawdust and were given acidified tap water and nonsterilized food pellets (type AM-II; Hope Farms, Woerden, The Netherlands) ad libitum. Cages and sawdust were replaced twice weekly. No special measures were taken to prevent coprophagia. The effect of parenteral treatment with once-daily doses of the cephalosporins under study was assessed over 9 to 11 days, the exact duration depending on the conditions of the individual experiments.

Effect of cephalosporins on number of aerobic gram-negative rods in feces of conventional mice. The mice were given one daily subcutaneous injection of the drug under study at various dosages on nine consecutive days. For all experiments, the dose ranges studied were 10 to 160 mg/kg/day for ceftazidime, 6.25 to 100 mg/kg/day for cefepime, 25 to 400 mg/kg/day for cefoperazone, and 5 to 80 mg/kg/day for ceftriaxone. These experiments used five mice each.

* Corresponding author.

On each weekday, fresh fecal pellets were collected, weighed, and suspended in 1 ml of PBS. Tenfold serial dilutions of these suspensions were made. Samples (0.1 ml) of the dilutions were inoculated on both blood agar plates and MacConkey III agar plates (Oxoid, Basingstoke, United Kingdom) which were incubated at 37°C. After incubation, differential counts were made on the MacConkey agar plates. The lower limit of detection was 500 CFU of aerobic gram-negative rods per g of feces. If no aerobic gram-negative rods were recovered from the feces, the number of CFU per gram of feces was arbitrarily taken as 100 for further calculations. Results are expressed as the geometric mean of the number of aerobic gram-negative rods per gram of feces.

Identification of members of the family *Enterobacteriaceae* was based on Gram staining, absence of oxidase production, and biotyping by the API 20E system (API Laboratories, La Balme, France).

In addition, the MICs of the cephalosporins for the isolated strains of aerobic gram-negative rods were determined by an agar dilution method (4), with Mueller-Hinton agar (Oxoid) as the test medium and an inoculum density of between 5×10^3 and 5×10^4 CFU per spot.

Effect of cephalosporins on number of *Candida albicans* in feces of postnatally contaminated mice. The effect of the cephalosporins on the outgrowth of *Candida albicans* (strain UC-820) was assessed in a murine model of gastrointestinal candidiasis in a separate group of mice, as described by Pope et al. (21). Six-day-old mice were orally contaminated with 2×10^6 to 5×10^6 CFU of *Candida albicans*. At the age of 21 to 22 days, the mice were weaned and housed in groups of four. Each treatment group consisted of eight mice. Treatment with the cephalosporins was started on day 1 after weaning and consisted of a single subcutaneous dose of the drug per day. Control mice received PBS. Fresh feces were collected before and various days after the start of treatment. These samples were weighed, suspended, and diluted in PBS, after which the number of *Candida albicans* per gram of feces was determined by quantitative culturing on Sabouraud agar (Oxoid) supplemented with 20 mg of kanamycin (Gist-Brocades, Delft, The Netherlands) per liter and BIGGY (bismuth sulfite glucose glycine yeast) agar (Oxoid). Plates were incubated at 37°C for 48 h, and the CFU were counted. The lower limit of detection of this assay was 500 CFU of *Candida albicans* per g of feces.

Oral contamination with *Citrobacter freundii* and effect of cephalosporins on recovery of this microorganism from feces of mice. The effect of each of the cephalosporins on the outgrowth of a resistant strain of *Citrobacter freundii* (MIC: ceftazidime, 128 μg/ml; ceftriaxone, 64 μg/ml; cefoperazone, 64 μg/ml; and cefepime, 0.25 μg/ml) was assessed after daily oral administration of an inoculum of 5×10^7 CFU of *Citrobacter freundii* through a feeding needle for 7 days. This strain of *Citrobacter freundii*, chosen from a panel of clinical isolates of gram-negative bacteria resistant to broad-spectrum cephalosporins (kindly donated by J. van der Klundert), displayed adequate colonizing properties in the gastrointestinal tract of mice combined with the highest level of resistance to the cephalosporins under study. This experiment was performed in a separate group of mice. Each treatment group consisted of six mice. These mice harbored no cephalosporin-resistant aerobic gram-negative rods in their feces before the experiment. Treatment with a cephalosporin and oral contamination with *Citrobacter freundii* were begun on the same day. The mice were given the drug subcutaneously for 11 days. The number of *Citrobacter freundii* per gram of feces was determined by quantitative culturing on MacConkey III agar plates supplemented with 1 μg of ceftazidime per ml. The plates were incubated at 37°C for 24 h, after which the CFU were counted. The lower limit of detection of this assay was 500 CFU of *Citrobacter freundii* per g of feces. If no *Citrobacter freundii* was recovered from the feces, the number of CFU per gram of feces was arbitrarily taken as 100 for further calculations. Results are expressed as the geometric mean of the number of *Citrobacter freundii* per gram of feces.

Effect of cephalosporins on relative cecal weight and total number of bacteria in the cecum. The relative cecal weight (16) and the total number of bacteria per gram of cecum were determined after 10 days of parenteral treatment with a cephalosporin. For these determinations, mice were killed by cervical dislocation, the cecum was removed and weighed, and the total wet weight was expressed as milligrams per gram of body weight. For the total count of bacteria per gram of cecal homogenate, the excised cecum was homogenized in 2 ml of sterile ice-cold PBS, the homogenate was diluted in PBS, and the total number of bacteria in the appropriate dilutions was determined on Gram-stained slides and compared with an external standard by the method of Holdeman and Moore (11). This count reflects the number of anaerobic bacteria in the cecum (12, 17).

Pharmacokinetics of cephalosporins in cecal contents of mice. Single-dose pharmacokinetic studies were performed for the following subcutaneous doses: cefoperazone, 100 mg/kg; ceftazidime, 160 mg/kg; ceftriaxone, 5 mg/kg; and cefepime, 100 mg/kg. At various time points after administration, mice were killed by exposure to 100% CO_2, the cecum was excised, and the cecal contents were removed, weighed, and diluted in 2 ml of PBS. This material was centrifuged at $1,500 \times g$ for 10 min at room temperature, after which the concentrations in the supernatant were determined by high-performance liquid chromatography (HPLC). The area under the concentration-time curve (AUC) was calculated according to the trapezoidal rule.

HPLC analysis of cephalosporin concentrations. Acetonitrile, dichloromethane, and sodium acetate were of analytical grade and were supplied by Merck (Darmstadt, Federal Republic of Germany). The liquid chromatographic system consisted of a constant-flow pump (model 1000; Sykam, Analytica BV, Rijswijk, The Netherlands), a Rheodyne model 7125 injection valve equipped with a 20-μl sample loop (Chrompack, Middelburg, The Netherlands), a stainless-steel column (length, 10 cm, and internal diameter, 3 mm), and a Spectroflow 773 absorbance detector (Kipp & Zn., Delft, The Netherlands). For monitoring, the detector was set at 254 nm for ceftazidime, 274 nm for ceftriaxone, 254 nm for cefoperazone, and 280 nm for cefepime. The column was home-packed with 5-μm Hypersil ODS (Shandon SPL, Cheshire, United Kingdom) according to a pressurized-slurry technique.

The extraction procedure applied to the samples containing cefoperazone, ceftazidime, or ceftriaxone was as follows. A 500-μl aliquot of the sample was vigorously mixed with an equal volume of acetonitrile in a polypropylene tube on a whirlmixer for 30 s. After centrifugation for 5 min at $1,200 \times g$, 400 μl of the supernatant was mixed with 3 ml of dichloromethane for 30 s and centrifuged for 5 min at $1,200 \times g$. A 20-μl aliquot of the upper aqueous phase containing the analyte was injected onto the column and chromatographed at a flow rate of 1 ml/min with a mobile phase consisting of 0.7% (vol/vol) acetonitrile in a 0.005-mol/liter

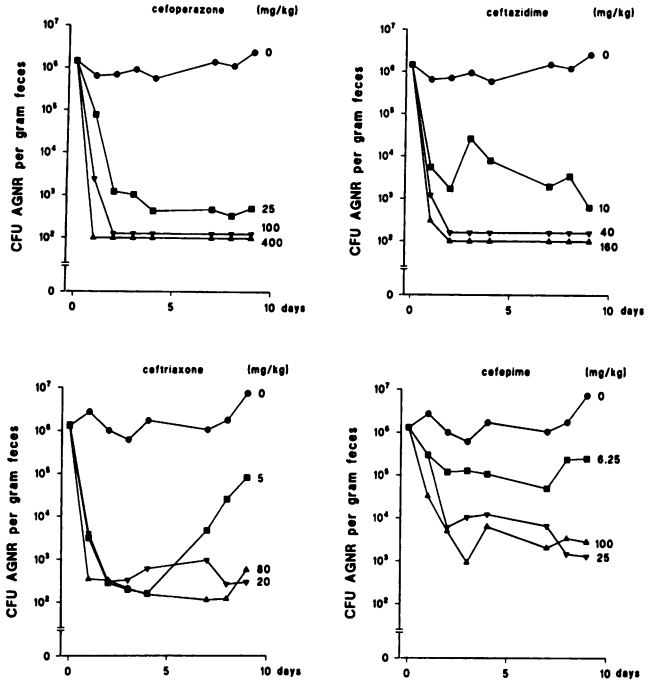

FIG. 1. Effect of parenteral treatment with each of four cephalosporins for 9 days on the total number of indigenous aerobic gram-negative rods (AGNR) in the feces of mice. Each symbol represents the geometric mean of the number of aerobic gram-negative bacteria per gram of feces of five mice. The numbers at the end of the curve give the dose of the drug administered daily.

acetate buffer (pH 5.5). Samples containing cefepime were extracted by the method of Barbhaiya et al. (3). Calibration plots were obtained by assaying samples of pooled supernatants of murine cecal contents, spiked in the range between 0.5 and 100 μg/ml.

Statistical procedures. The results pertaining to the quantitative fecal cultures are given as the geometric mean of the number of CFU per gram of feces. Other results are expressed as mean ± standard error. Repeated-measures analysis of variance was used for statistical comparisons (7). A P level of 0.05 was considered significant.

RESULTS

Effect of cephalosporins on number of aerobic gram-negative rods in feces of conventional mice. The effect of 9 to 10 days of treatment with one daily subcutaneous dose of cefoperazone, ceftazidime, ceftriaxone, or cefepime on the total number of aerobic gram-negative rods in the feces is shown in Fig. 1. All four cephalosporins reduced the number of aerobic gram-negative rods in the feces, but cefepime was less effective in this respect than cefoperazone, ceftazidime, or ceftriaxone. The results suggested that complete eradication of aerobic gram-negative rods within 1 week was achieved by cefoperazone at doses of 100 mg/kg/day and higher, by ceftazidime at 40 mg/kg/day and higher, and by ceftriaxone at 80 mg/kg/day.

The gram-negative species isolated from the feces before the start of treatment were *Escherichia coli*, *Proteus mirabilis*, and *Morganella morganii*, among which *E. coli* predominated (usually accounting for more than 90%). The aerobic gram-negative rods still present in the feces after exposure to low doses of any of the cephalosporins were *E. coli* and *Proteus* spp. The MICs of the four cephalosporins for the isolated strains of aerobic gram-negative rods are given in Table 1. No change in the antimicrobial susceptibility of the isolated bacteria to any of the four cephalosporins was detected during the study. Colonization with new biotypes of gram-negative species during this period was not observed either.

Effect of cephalosporins on number of *Candida albicans* in feces of postnatally contaminated mice. To determine whether parenteral treatment with broad-spectrum cephalosporins predisposes to intestinal colonization and overgrowth with *Candida albicans*, we administered the drugs to mice contaminated postnatally with *Candida albicans*. In the control mice, which were treated with PBS from day 21 on, the number of *Candida albicans* tended to decrease slightly during the study period, i.e., from between 10^5 and 10^6 CFU/g of feces at the start to between 10^4 and 10^5 10 days later (Fig. 2). A dose of 400 mg of cefoperazone per kg per day led to a significant increase in the numbers of *Candida albicans* within 2 days after the start of treatment to between 10^7 and 10^8 CFU/g of feces. Treatment with 80 mg of ceftriaxone per kg per day had a smaller, but still significant positive effect on the outgrowth of *Candida albicans*. In mice given all other dosages of cefoperazone, ceftriaxone, ceftazidime, or cefepime, the outgrowth of *Candida albicans* did not differ significantly from that in controls.

Oral contamination with *Citrobacter freundii* and effect of cephalosporins on recovery of this microorganism in feces of mice. To determine whether treatment with the cephalosporins could lead to colonization with resistant gram-negative rods, we contaminated adult mice orally with a resistant strain of *Citrobacter freundii*. In the control mice, the geometric mean number of these bacteria during week 1 of the experiment lay between 10^5 and 10^6 CFU/g of feces (Fig. 3). In week 2 of the experiment, the oral contamination was discontinued and the number of CFU of *Citrobacter freundii* tended to fall to levels below the detection limit (500 CFU/g of feces). For statistical purposes, these mice were arbitrarily considered to have 100 CFU of *Citrobacter freundii* per g in their feces (Fig. 3).

In cephalosporin-treated mice, the administration of the drug and oral contamination with *Citrobacter freundii* were begun simultaneously. Treatment with 400 mg of cefoperazone per kg per day had the strongest effect on the microbial ecology of the intestinal tract, resulting in an increase of the

TABLE 1. MICs of the cephalosporins for cultured aerobic gram-negative rods from feces of mice

Organism	MIC (mg/liter)			
	Cefoperazone	Ceftazidime	Ceftriaxone	Cefepime
Escherichia coli	0.125–0.25	0.063–0.25	0.032–0.063	0.016–0.032
Proteus mirabilis	1	0.063	0.008–0.016	0.032
Morganella morganii	0.5 –1	0.063	0.004–0.032	0.016

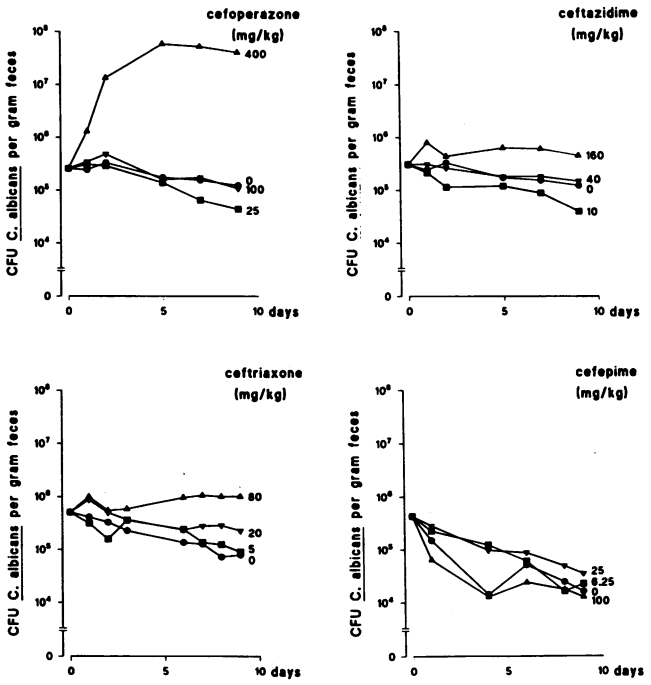

FIG. 2. Effect of 9 to 10 days of parenteral treatment with the four cephalosporins under study on the recovery of *Candida albicans* in the feces of mice. Each symbol represents the geometric mean of the number of *Candida albicans* per gram of feces of eight mice. The numbers at the end of the curve give the dose of the drug administered daily.

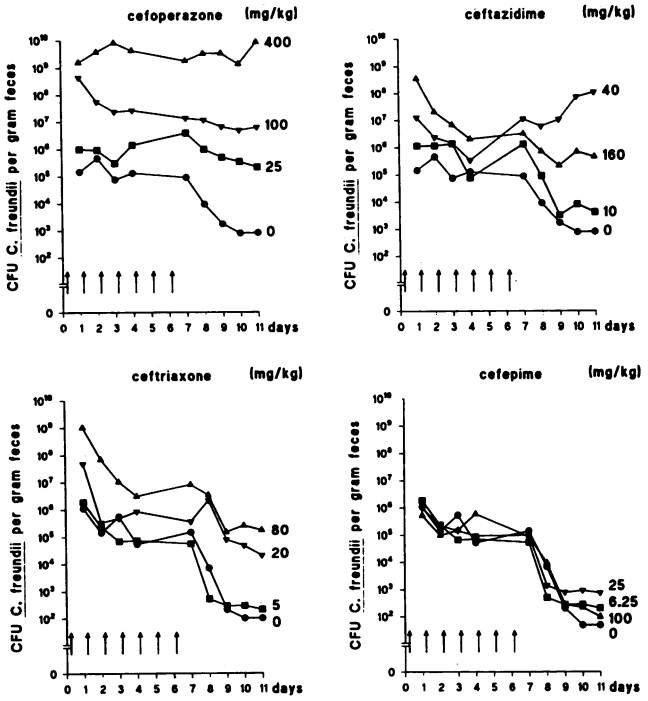

FIG. 3. Effect of parenteral treatment with each of the four cephalosporins for 11 days on the recovery of *Citrobacter freundii* in the feces of mice during daily oral contamination of the mice with 5×10^7 CFU for the first 7 days. Each symbol represents the geometric mean of the number of *Citrobacter freundii* per gram of feces of six mice. The numbers at the end of the curve give the dose of the drug administered daily. The arrows indicate the times of contamination of the mice with *Citrobacter freundii*.

number of *Citrobacter freundii* to between 10^9 and 10^{10} CFU/g of feces (Fig. 3). Cefoperazone at lower dosages, 40 mg or more of ceftazidime per kg, or 20 mg or more of ceftriaxone per kg daily had less pronounced effects on the numbers of *Citrobacter freundii* in the feces, which increased to between 10^6 and 10^8 CFU/g of feces. Treatment with cefepime (up to 100 mg/kg/day) did not have any significant effect on the recovery of this strain (Fig. 3), but this strain was susceptible to cefepime (MIC, 0.25 μg/ml).

Effect of cephalosporins on relative cecal weight and total number of bacteria in the cecum. The relative cecal weight is another parameter of colonization resistance, and the total number of bacteria in the cecum reflects the number of anaerobes in that organ. Administration of 400 mg of cefoperazone per kg per day or 20 or 80 mg of ceftriaxone per kg per day led to a significant increase in the relative cecal weight (Table 2). Only 400 mg of cefoperazone per kg per day gave a significant decrease in the total number of bacteria in the cecum (Table 2). Other treatments had no effect on either the relative cecal weight or the total number of bacteria in the cecum.

Pharmacokinetics of cephalosporins in cecal contents in mice. The concentrations of the cephalosporins in the contents of the cecum are shown in Fig. 4. The AUC from 0 to 5 h was 4,548 μg · h/g for a dose of 100-mg/kg cefoperazone, 583 μg · h/g for 160-mg/kg ceftazidime, 68.8 μg · h/g for 5-mg/kg ceftriaxone, and 20.5 μg · h/g for 100-mg/kg cefepime. The ratios between the AUC and the dose, as relative parameters of the proportion of the dose excreted into the gastrointestinal tract, were 45.5 for cefoperazone, 3.64 for ceftazidime, 13.8 for ceftriaxone, and 0.16 for cefepime.

To estimate the percentage of a parenteral dose excreted into the gastrointestinal tract, we also measured concentrations of ceftazidime in cecal contents after oral administration. The AUC from 0 to 5 h (AUC_{0-5}) for cecal contents after an oral dose of 160 mg of ceftazidime per kg was 19,420 μg · h/g. If it is correct to assume that orally administered ceftazidime is not absorbed or metabolized in the gastroin-

TABLE 2. Effect of 10 days of treatment with cephalosporins on the relative cecal weight and the total number of bacteria per gram of cecal homogenate

Drug	Dose (mg/kg)	Relative cecal weight (mg/g of body wt)[a]	Log no. of bacteria/g of cecum[a]
Control		16.3 (3.0)	10.9 (0.2)
Cefoperazone	400	41.3 (12.2)[b]	8.8 (1.1)[b]
	100	19.9 (5.5)	10.7 (0.6)
	25	18.4 (4.6)	11.0 (0.2)
Ceftazidime	160	19.0 (3.0)	10.9 (0.2)
	40	16.7 (2.9)	11.0 (0.2)
	10	14.8 (2.8)	11.1 (0.1)
Ceftriaxone	80	22.9 (3.4)[b]	10.8 (0.2)
	20	21.9 (5.5)[b]	10.8 (0.3)
	5	15.2 (2.4)	11.1 (0.2)
Cefepime	100	17.2 (2.1)	11.0 (0.1)
	25	15.3 (2.7)	11.0 (0.1)
	6.25	15.1 (2.3)	11.1 (0.2)

[a] Values in parentheses give the standard deviation.
[b] Significantly different from the control mice ($P < 0.05$).

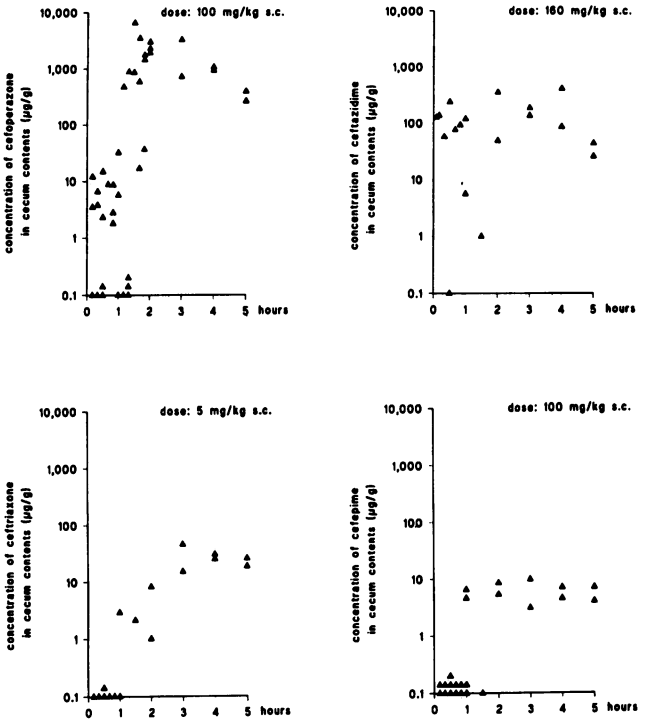

FIG. 4. Drug concentrations in cecal contents of mice given a single subcutaneous (s.c.) dose of cefoperazone, ceftazidime, ceftriaxone, or cefepime. Each symbol represents the measured value in a single mouse.

testinal tract of mice, this value can be used to estimate the fraction of the subcutaneous (s.c.) dose of a drug that is excreted into the gastrointestinal tract, according to the following equation: $F = AUC_{s.c.}/AUC_{oral}$, where F is the excreted fraction. For a subcutaneous dose of 160 mg of ceftazidime per ml, this value was 0.03, which means that 3% of a subcutaneously administered dose of ceftazidime is excreted into the gastrointestinal tract. Using this estimate for the other three drugs, we calculated the proportion of the dose excreted into the gastrointestinal tract, which was 36.5% for cefoperazone, 11.1% for ceftriaxone, and 0.16% for cefepime. These percentages should be considered rough estimates of the true percentage of the dose that is excreted into the gastrointestinal tract of mice.

DISCUSSION

The results of the present study showed that parenteral administration of broad-spectrum cephalosporins can have a major ecological impact on the intestinal flora of mice, since the investigated drugs (cefoperazone, ceftazidime, cefepime, and ceftriaxone) caused a marked dose-dependent decrease of the number of aerobic gram-negative rods. It may be argued that this decrease might even be more if coprophagia would have been prevented. However, this does not mean that the effects of the various dosage regimens are not comparable, because the mice were always housed in a closed system under identical conditions. Nevertheless, the coprophagic behavior of mice has to be borne in mind if the results in mice are to be extrapolated to the human situation.

Other ecological effects of parenteral treatment with the cephalosporins were observed as well. In mice orally contaminated with a resistant strain of *Citrobacter freundii*, the number of these organisms in the feces increased during parenteral administration of cefoperazone, ceftazidime, and ceftriaxone but not during administration of cefepime. It is noteworthy that this outgrowth of *Citrobacter freundii* occurred despite the fact that the concentrations of the cephalosporins in the cecal contents were much higher than the MIC. For example, the outgrowth of the *Citrobacter* strain in the feces increased with the dose of cefoperazone up to 400 mg/kg/day. At this dose of cefoperazone, the concentrations in cecal contents can be expected to be as high as 40,000 μg/g, whereas the MIC of cefoperazone for this strain was only 64 μg/ml. Therefore, this strain of *Citrobacter freundii* can be considered resistant in vivo (except for cefepime), and therefore it is a suitable indicator strain for the detection of a decrease of colonization resistance.

Parenteral administration of high doses of cefoperazone and ceftriaxone also led to an increase of the outgrowth of *Candida albicans* and an increase of the relative cecal weight of mice, but only high doses of cefoperazone gave a marked decrease of the total number of bacteria in the cecum. An increase of the relative cecal weight is generally observed in mice with a disturbed intestinal microflora (17) and has been shown to correlate with a decrease in colonization resistance of the gastrointestinal tract (16).

Colonization resistance of the gastrointestinal tract has classically been linked to an intact anaerobic microflora (26). Until now, the question of which anaerobic bacteria are important in maintaining colonization resistance and whether aerobic bacteria can play a role in this process as well is unsolved. The main problem in addressing these questions is the culturing and identification of murine intestinal anaerobes. Despite laborious anaerobic culture methods, no one has yet succeeded in culturing more than 60 to 80% of the anaerobic intestinal bacteria of mice (12, 18). In the present study, we decided to count the anaerobic bacteria indirectly via a microscopical method (11, 17). Since anaerobic bacteria account for more than 90% of the flora of the mouse cecum (19), the decrease in the total number of bacteria implies a decrease in the number of anaerobic bacteria in the cecum.

Various parameters of disturbed colonization resistance in mice treated with broad-spectrum cephalosporins can be compared on the basis of the present results. A standard method used to establish a decrease of colonization resistance is measurement of the outgrowth of a resistant aerobic gram-negative rod in the feces of mice during treatment with antimicrobial agents. In this study, *Citrobacter freundii* was used as the test strain because it combines two characteristics: low sensitivity to three of the four cephalosporins under study and adequate recovery from the feces of mice. Increased outgrowth of *Citrobacter freundii* in the feces occurred at low doses of ceftazidime and cefoperazone, whereas at these dosages all other parameters of decreased colonization resistance, i.e., outgrowth of *Candida albicans* in feces, relative cecal weight, and total number of bacteria in the cecum, showed no deviation from the controls (Table 3). This led us to conclude that the latter three parameters are less sensitive for the detection of reduced colonization resistance than the outgrowth of *Citrobacter freundii* in the feces of mice treated with broad-spectrum cephalosporins is.

The ecological changes of the intestinal flora of mice by parenteral treatment with cephalosporins described in the present study can also be expected to occur in humans. The concentrations of cefoperazone in the feces of a group of four patients treated with 4 g of cefoperazone per day varied

TABLE 3. Comparison of the various effects of the cephalosporins on the intestinal ecology of mice[a]

Drug	Dose (mg/kg)	Relative cecal wt	Total count of bacteria in cecum	Outgrowth of *Candida albicans* in feces	Outgrowth of *Citrobacter freundii* in feces
Cefoperazone	400	↑	↓	↑	↑
	100	=	=	=	↑
	25	=	=	=	↑
Ceftazidime	160	=	=	=	↑
	40	=	=	=	↑
	10	=	=	=	=
Ceftriaxone	80	↑	=	↑	↑
	20	↑	=	=	↑
	5	=	=	=	=
Cefepime	100	=	=	=	=[b]
	25	=	=	=	=[b]
	6.25	=	=	=	=[b]

[a] =, Not significantly different from control mice; ↑, significantly higher than in control mice; ↓, significantly lower than in control mice.

[b] The strain of *Citrobacter freundii* was susceptible to cefepime (MIC, 0.25 μg/ml), and therefore these results are difficult to interpret.

from 10,700 to 21,700 μg/g of feces (20). In another study, Silva et al. (24) found lower concentrations of cefoperazone (<20 to 15,430 μg/g) in the feces of volunteers treated three times with a dose of 1 or 2 g of cefoperazone, and in yet another study, Giuliano et al. (10) found concentrations of cefoperazone in the feces ranging from 2,727 to 8,840 μg/g in a group of four volunteers receiving 4 g of cefoperazone per day. Since only 19 to 36% of a parenteral dose of cefoperazone is excreted renally and considerable biliary excretion of cefoperazone has been documented (6), high concentrations of cefoperazone in the feces during parenteral treatment with this drug are not surprising.

Arvidsson et al. (2) measured biliary excretion of ceftriaxone during the steady state in five healthy male volunteers. They estimated that 11 to 65% of the parenteral dose of ceftriaxone was excreted into the biliary tract. In these individuals, ceftriaxone concentrations were measured in the feces 1 day after they had received a total dose of approximately 500 mg of ceftriaxone. In two of these subjects, no ceftriaxone concentrations could be detected in the feces, whereas in the other three subjects, the ceftriaxone concentrations ranged from 28 to 75 μg/g. To our knowledge, there is no information on the concentrations of ceftazidime and cefepime in the feces of humans, but concentrations of these two drugs in the feces can be expected to be much lower than those of cefoperazone and ceftriaxone, because these antibiotics are mainly renally cleared.

The present study on the effects of cephalosporins on the gastrointestinal ecology in mice shows some similarities with those in humans. For example, Giuliano et al. (10) found that treatment of healthy volunteers with cefoperazone led to a dramatic increase in the number of yeasts in the feces, whereas treatment with cefoxitin had much less effect. The antibacterial spectra of the two drugs are similar, but the concentrations of cefoxitin in the feces were much lower than those of cefoperazone.

That the colonization resistance-disturbing potential of antibiotics is clinically relevant has never been proved conclusively, but a decrease of colonization resistance is believed to increase the chance of overgrowth of the gastrointestinal tract by multiresistant bacteria or yeasts. In patients with increased susceptibility to infection, intestinal overgrowth with potentially pathogenic microorganisms increases the chance of an infection with those organisms, especially if the mucosa of the gastrointestinal tract has been damaged by cytostatic agents or irradiation. This situation underscores the importance of knowing the therapeutic efficacy as well as the colonization resistance-disturbing effect of a given antibiotic. A study on both these effects could be expected to yield an indication of the relationship between the dosage at which colonization resistance is decreased and the dosage giving maximum therapeutic efficacy. In vitro cefepime and ceftriaxone have much lower MICs against members of the family *Enterobacteriaceae* than ceftazidime and cefoperazone (8, 13, 25). We have observed that the in vitro order of potency of these four cephalosporins is similar to the in vivo order of potency in an experimental thigh muscle infection with *E. coli* in irradiated mice (27), indicating that in therapeutic doses cefepime and ceftriaxone have less effect on colonization resistance than ceftazidime and cefoperazone. Although direct extrapolation to humans should be done cautiously, our results suggest that the margin of safety with respect to colonization resistance is greater for cefepime and ceftriaxone than for ceftazidime and cefoperazone.

ACKNOWLEDGMENTS

We thank A. M. Hazekamp-van Dokkum and E. van Strijen for their technical assistance and A. Zwinderman from the Department of Medical Statistics for statistical advice.

This work was supported by the J. A. Cohen Institute for Radiopathology and Radiation Protection.

REFERENCES

1. **Alestig, K., H. Carlberg, C. E. Nord, and B. Trollfors.** 1983. Effect of cefoperazone on faecal flora. J. Antimicrob. Chemother. **12**:163–167.
2. **Arvidsson, A., G. Alván, B. Angelin, O. Borgå, and C. E. Nord.** 1982. Ceftriaxone: renal and biliary excretion and effect on the colon microflora. J. Antimicrob. Chemother. **10**:207–215.
3. **Barbhaiya, R. H., S. T. Forgue, W. C. Shyu, E. A. Papp, and K. A. Pittman.** 1987. High-pressure liquid chromatographic analysis of BMY-28142 in plasma and urine. Antimicrob. Agents Chemother. **31**:55–59.
4. **Barry, A. L.** 1986. Procedure for testing antimicrobial agents in agar media: theoretical considerations, p. 1–26. *In* V. Lorian (ed.), Antibiotics in laboratory medicine, 2nd ed. Williams & Wilkins, Baltimore.
5. **Bodey, G. P., V. Fainstein, I. Garcia, B. Rosenbaum, and Y. Wong.** 1983. Effect of broad-spectrum cephalosporins on the microbial flora of recipients. J. Infect. Dis. **148**:892–897.
6. **Brogden, R. N., A. Carmine, R. C. Heel, P. A. Morley, T. M. Speight, and G. S. Avery.** 1981. Cefoperazone: a review of its in vitro antimicrobial activity, pharmacological properties and therapeutic efficacy. Drugs **22**:423–460.
7. **Edwards, A. L.** 1985. Multiple regression and the analysis of variance and covariance, 2nd ed., p. 115–129. W. H. Freeman & Co., New York.
8. **Fass, R. J.** 1983. Comparative in vitro activities of third-generation cephalosporins. Arch. Intern. Med. **143**:1743–1745.
9. **Forgue, S. T., W. C. Shyu, C. R. Gleason, K. A. Pittman, and R. H. Barbhaiya.** 1987. Pharmacokinetics of the novel cephalosporin cefepime (BMY-28142) in rats and monkeys. Antimicrob. Agents Chemother. **31**:799–804.
10. **Giuliano, M., M. Barza, N. V. Jacobus, and S. L. Gorbach.** 1987. Effect of broad-spectrum parenteral antibiotics on the composition of intestinal microflora of humans. Antimicrob. Agents Chemother. **31**:202–206.
11. **Holdeman, L. V., and W. E. C. Moore (ed.).** 1973. Anaerobe

laboratory manual, 2nd ed., p. 107. Virginia Polytechnic Institute and State University, Blacksburg.
12. **Itoh, K., and T. Mitsuoka.** 1985. Comparison of media for isolation of mouse anaerobic faecal bacteria. Lab. Anim. **19:** 353–358.
13. **Kessler, R. E., M. Bies, R. E. Buck, D. R. Chisholm, T. A. Pursiano, Y. H. Tsai, M. Misiek, K. E. Price, and F. Leitner.** 1985. Comparison of a new cephalosporin, BMY 28142, with other broad-spectrum β-lactam antibiotics. Antimicrob. Agents Chemother. **27:**207–216.
14. **Khan, N. J., J. A. Bihl, R. F. Schell, J. L. LeFrock, and S. J. Weber.** 1984. Antimicrobial activities of BMY-28142, cefbuperazone, and cefpiramide compared with those of other cephalosporins. Antimicrob. Agents Chemother. **26:**585–590.
15. **Klastersky, J.** 1989. Empiric treatment of infection during granulocytopenia: a comprehensive approach. Infection **17:**59–64.
16. **Koopman, J. P., F. G. J. Janssen, and J. A. M. Van Druten.** 1977. The relation between the intestinal microflora and intestinal parameters in mice. Z. Versuchstierkd. **19:**54–61.
17. **Koopman, J. P., H. M. Kennis, A. M. Stadhouders, and H. De Boer.** 1984. Selective elimination of Enterobacteriaceae species from the digestive tract in mice and rats. Z. Versuchstierkd. **26:**197–204.
18. **Koopman, J. P., G. W. Welling, A. W. M. Huybregts, J. W. M. A. Mullink, and R. A. Prins.** 1981. Association of germ-free mice with intestinal microflora. Z. Versuchstierkd. **23:**145–154.
19. **Lee, A., J. Gordon, C.-J. Lee, and R. Dubos.** 1971. The mouse intestinal microflora with emphasis on the strict anaerobes. J. Exp. Med. **133:**339–352.
20. **Mulligan, M. E., D. M. Citron, B. T. McNamara, and S. M. Finegold.** 1982. Impact of cefoperazone therapy on fecal flora. Antimicrob. Agents Chemother. **22:**226–230.
21. **Pope, L. M., G. T. Cole, M. N. Guentzel, and L. J. Berry.** 1979. Systemic and gastrointestinal candidiasis of infant mice after intragastric challenge. Infect. Immun. **25:**702–707.
22. **Richards, D. M., and R. N. Brogden.** 1985. Ceftazidime: a review of its antibacterial activity, pharmacokinetic properties and therapeutic use. Drugs **29:**105–161.
23. **Richards, D. M., R. C. Heel, R. N. Brogden, T. M. Speight, and G. S. Avery.** 1984. Ceftriaxone: a review of its antibacterial activity, pharmacological properties and therapeutic use. Drugs **27:**469–527.
24. **Silva, M., N. A. Cornick, and S. L. Gorbach.** 1989. Suppression of colonic microflora by cefoperazone and evaluation of the drug as potential prophylaxis in bowel surgery. Antimicrob. Agents Chemother. **33:**835–838.
25. **Tsuji, A., A. Maniatis, M. A. Bertram, and L. S. Young.** 1985. In vitro activity of BMY-28142 in comparison with those of other β-lactam antimicrobial agents. Antimicrob. Agents Chemother. **27:**515–519.
26. **Van der Waaij, D., J. M. Berhguis-de Vries, and J. E. C. Lekkerkerk-van der Wees.** 1971. Colonization resistance of the digestive tract in conventional and antibiotic-treated mice. J. Hyg. **69:**405–411.
27. **Van Ogtrop, M. L., H. Mattie, H. F. L. Guiot, E. van Strijen, A. M. Hazekamp-van Dokkum, and R. van Furth.** 1990. Comparative study of the effects of four cephalosporins against *Escherichia coli* in vitro and in vivo. Antimicrob. Agents Chemother. **34:**1932–1937.