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Effects on Oral and Intestinal Microfloras of Norfloxacin and Pefloxacin for Selective Decontamination in Bone Marrow Transplant Patients

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We monitored the modifications of oral and intestinal microfloras of 10 allogeneic bone marrow recipients who received randomly either norfloxacin or pefloxacin (400 mg three times a day) as selective decontamination for infection prevention. After 1 week of treatment, in all patients members of the family *Enterobacteriaceae* were no longer detectable and in all but one pefloxacin-treated patient enterococci were also eliminated in the intestine. The anaerobic flora was not affected, with the exception of *Bacteroides* spp., markedly reduced after treatment with pefloxacin. In most patients the most striking effect was the increase in staphylococcal counts. These strains were found to be resistant to both quinolones in the study. Less consistent changes were observed in oral flora. No relevant difference could be demonstrated between the two regimens on bacterial counts either in feces or in saliva. This study shows the efficacy of both quinolones in eradicating gram-negative bacilli in the alimentary tract of bone marrow transplant patients; however, the finding of the overgrowth of resistant gram-positive organisms during treatment with these agents deserves further evaluation.

Infection is the most frequent complication and a major cause of death in patients with profound and prolonged granulocytopenia (11). The principle of selective decontamination of the gastrointestinal tract has been applied successfully to reduce endogenous infection in severely immunocompromised patients: oral antibiotics are used to eliminate the aerobic potentially pathogenic gram-negative organisms in the intestine while preserving the normally predominant anaerobic flora (2, 7, 8, 16, 24).

Fluoroquinolones, a new class of antibiotics (9, 23) highly active against aerobic gram-negative bacilli, are currently being tested as selective prophylactic regimens during neutropenia. Studies with norfloxacin have been carried out and have demonstrated its efficacy in eliminating gram-negative rods from the intestine of treated patients while sparing the other components of the microflora; this effect was reflected by a decrease in the morbidity associated with gram-negative infections (12, 21). Pefloxacin, one of the newer fluoroquinolones, has a better systemic absorption compared with norfloxacin (22) and could have a prophylactic effect also against bacterial infections originating outside the gastrointestinal tract (e.g., the oral cavity); furthermore, because of the broader spectrum of activity of pefloxacin (3), it might also be a better agent for preventing infections caused by gram-positive organisms.

Therefore, we carried out a comparative microbiological study with a group of severely immunocompromised patients as allogeneic bone marrow recipients who received either norfloxacin or pefloxacin as antibiotic prophylaxis. We evaluated the efficacy of the two quinolones in decontaminating the alimentary tract by monitoring the modifications of oral and intestinal microfloras and the emergence of resistant strains.

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MATERIALS AND METHODS

Patient population and antimicrobial prophylaxis. Ten patients who received allogeneic bone marrow grafts for the treatment of either acute leukemia or chronic myeloid leukemia participated in this study. The conditioning regimen included cyclophosphamide and total body irradiation in all patients. Graft-versus-host disease prophylaxis was performed with cyclosporin A. All patients had a central venous line inserted. They were nursed in single bedrooms and received reverse isolation precautions. They were randomly assigned to receive orally either norfloxacin (400 mg three times a day) or pefloxacin (400 mg three times a day) starting 1 day before transplant. Oral antifungal prophylaxis with amphotericin B (3 g/day) was administered to all patients. Prophylaxis was discontinued when the granulocyte count increased above 1,000/μl.

Collection and processing of specimens. Saliva and fecal samples were collected 24 to 48 h before antibiotic prophylaxis and after 1 week of administration. All specimens were stored in brain heart infusion broth plus 10% glycerol at −20°C until processed (within 1 week). An analysis of specimens was performed as previously described (6) with minor modifications. Samples of 0.1 ml of selected dilutions in phosphate-buffered saline were plated on the following media for the purposes indicated: Columbia blood agar with vitamin K and hemin for total aerobic and anaerobic counts; MacConkey agar for the enumeration of members of the family *Enterobacteriaceae*; kanamycin-esculin-azide agar for enumeration of enterococci; salt-mannitol agar for staphylococci; pseudomonas agar with C-N supplement (200 mg of cetrimide and 15 mg of nalidixic acid per liter) for *Pseudomonas* spp.; mitis salivarius agar for oral streptococci; Mycosel agar for yeasts; kanamycin-vancomycin-laked blood agar for *Bacteroides* spp.; egg yolk agar (etha-

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TABLE 1. Patient characteristics

Characteristic	Norfloxacin	Pefloxacin
No.	5	5
Male/female (no.)	5/0	3/2
Age (mean yr ± SD)	34.2 ± 9.5	32.2 ± 8.3
Underlying illness (no.)		
Acute lymphocytic leukemia	1	0
Acute nonlymphocytic leukemia	2	4
Chronic myeloid leukemia	2	1
Mean days on study (±SD)	23.2 ± 8.5	18.6 ± 2.5
Duration of neutropenia (mean days ± SD)		
<100	3.7 ± 3.1	3.4 ± 1.8
100–499	11.6 ± 2	10.8 ± 2.3
500–999	6.8 ± 5.1	3.2 ± 0.4
No. of febrile episodes	5	3
Microbiologically documented infections (bacteremia) (no.)	4	0
Graft-versus-host disease (no.)		
Grade I	1	2
Grade II	3	
Survival (no.)	5	5

nol-treated dilutions) for *Clostridium* spp.; and cycloserin-cefoxitin-fructose agar for *Clostridium difficile*. All media and supplements were obtained from Oxoid, Basingstoke, Hampshire, England, with the exception of Mycosel agar, which was purchased from BBL Microbiology Systems, Cockeysville, Md., and mitis salivarius agar, which was provided by Difco Laboratories, Detroit, Mich. Plates were incubated at 37°C for 24 h for aerobic cultures or for 48 h in an anaerobic chamber for anaerobic cultures. Plates that showed 30 to 300 colonies were used for bacterial counts. By this method the lowest limit of detection was 50 organisms per g of feces or per ml of saliva. Staphylococcal isolates were further identified with the API Staph test kit (Analytab Products, La Balme Les Grottes, France). A change in bacterial counts of at least 2 $\log_{10}$ was considered meaningful with our methods.

Emergence of resistance. For enumeration of colonies of resistant aerobic microorganisms, samples were plated on Columbia blood agar containing 1 μg of norfloxacin per ml and 4 μg of pefloxacin per ml, respectively. MICs were determined for staphylococci by a macrodilution method in Mueller-Hinton broth (17). The following antibiotics were assayed: norfloxacin (Merck Sharp & Dohme, West Point, Pa.), pefloxacin (Rhone-Poulenc), ciprofloxacin (Bayer), oxacillin (Bristol Laboratories, Syracuse, N.Y.), imipenem (Merck Sharp & Dohme), gentamicin (Schering Corp., Bloomfield, N.J.), and vancomycin (Eli Lilly & Co., Indianapolis, Ind.).

RESULTS

Patient characteristics. The clinical data of the patients included in this study are shown in Table 1. The two groups of patients were similar in age, underlying disease, and duration of profound granulocytopenia. No patient had an adverse reaction to either drug. Four microbiologically documented infections (bacteremia) occurred in three patients in the norfloxacin group: the offending pathogens were *Staphylococcus epidermidis* in one patient, *Achromobacter xylosoxidans* in another patient, and *Corynebacterium* group JK and *Pseudomonas stutzeri* in two episodes in the third patient. In the pefloxacin group, three episodes of unexplained fever occurred; in two of these cases, graft-versus-host disease was diagnosed. All febrile patients received imipenem (40 mg/kg per day) as empiric antibiotic therapy for a mean period of 7 days (range, 3 to 10 days). In two cases, imipenem therapy was discontinued for the persistence of fever, and ceftazidime (8 g/day) and vancomycin (2 g/day) were substituted for 10 and 15 days, respectively. In one case (*S. epidermidis*) the pathogen isolated before imipenem therapy was resistant to both norfloxacin and pefloxacin. All patients showed primary bone marrow engraftment and were discharged from the hospital after 4 to 6 weeks.

Effects on intestinal and oral floras. The effects of intestinal microflora of norfloxacin- and pefloxacin-treated patients are shown in Fig. 1. In the feces of all norfloxacin-treated patients, both *Enterobacteriaceae* and enterococcal counts decreased under detectable limits after treatment. Staphylococcal strains were recovered before norfloxacin treatment in four patients and were all susceptible to the drug; after treatment, staphylococci were present in four patients and were all resistant to norfloxacin; in two cases, staphylococci increased markedly (mean increase, 3 logs). The total anaerobic count and the two genera searched for (*Bacteroides* and *Clostridium*) showed no difference in counts before and after treatment with norfloxacin (decrease, <2 logs). Pefloxacin caused a reduction in *Enterobacteriaceae* to undetectable levels in the feces of the five patients; enterococci were eliminated in all but one patient who had high counts of enterococci showing resistance to pefloxacin. Before pefloxacin treatment only one patient harbored staphylococci susceptible to the drug, while after treatment staphylococci were recovered in all pefloxacin-treated patients (mean count increase, 2.6 logs) and in four of five cases the staphylococci were resistant to the drug; in two patients staphylococci increased markedly (3.5 logs). Among anaerobes, *Bacteroides* spp. showed the most important change with pefloxacin (mean count decrease, 4 logs).

The effects on the oral flora are shown in Fig. 2. Less evident modifications were detected with both drugs: a mild decrease in the total anaerobic count (2.6 logs) and in *Bacteroides* counts (2.2 logs) was observed with pefloxacin; two norfloxacin- and two pefloxacin-treated patients had increased counts of staphylococci resistant to the drug used. Oral streptococci were not affected by either drug.

The changes in the microflora, observed after 1 week of treatment, were found to be quite stable since samples taken after 3 weeks of treatment from patients who had not received other antibiotics showed similar results (data not shown). One patient harbored *Pseudomonas putida* in the first fecal sample which was eradicated after administration of pefloxacin. No other *Pseudomonas* sp. was isolated in the feces or saliva of the patients studied. One pefloxacin-treated patient had a gram-positive coccus tentatively identified as *Leuconostoc* sp. resistant to quinolones and to vancomycin present in feces and saliva. During treatment with pefloxacin, one patient was colonized by *C. difficile* and had cytotoxin (titer, 1:20) in feces without complaining of

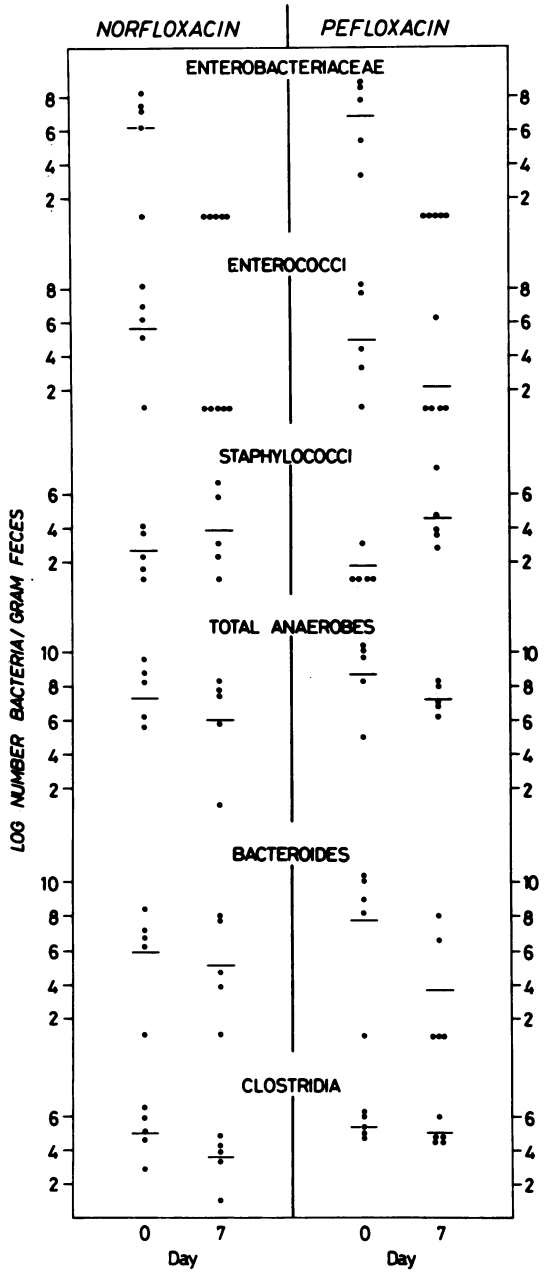

FIG. 1. Effects of norfloxacin and pefloxacin on the intestinal flora. Bar = geometric mean of counts of the five patients. Counts of below 1.7 $\log_{10}$ were not detectable.

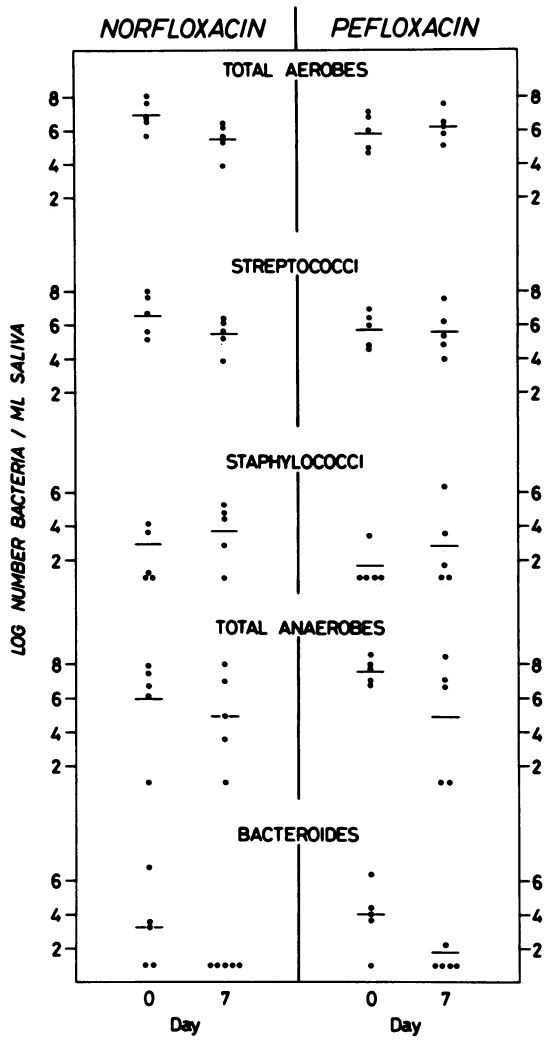

FIG. 2. Effects of norfloxacin and pefloxacin on the oral flora. Bar = geometric mean of counts of the five patients. Counts of below 1.7 $\log_{10}$ were not detectable.

diarrhea. No fungal colonization was observed in either group.

Emergence of resistant staphylococci. Staphylococci grown on media containing norfloxacin or pefloxacin were further analyzed. Eight strains coming from the fecal samples of seven different patients (four norfloxacin treated and three pefloxacin treated) were identified as *S. aureus* (one strain), *S. epidermidis* (four strains), *S. hominis* (two strains), and *S. haemolyticus* (one strain). MICs of different antimicrobial agents for these strains were also determined (Table 2). Cross resistance to the three quinolones tested was found: most strains required a MIC of >32 μg/ml for norfloxacin, ≥16 μg/ml for pefloxacin, and ≥16 μg/ml for ciprofloxacin; seven strains were resistant to gentamicin (MIC, >8 μg/ml), four strains were also resistant to oxacillin (MIC, >2 μg/ml), and two were resistant to imipenem (MIC, >8 μg/ml); vancomycin was active against all strains. One of the above-mentioned strains of *S. epidermidis*, present in high counts in the feces of one norfloxacin-treated patient, showed a pattern of resistance similar to that shown by the *S. epidermidis* strain causing bacteremia in the same patient.

TABLE 2. MICs of various antibiotics for coagulase-negative staphylococcal isolates

Antimicrobial agent	MIC (μg/ml)[a]	
	Range	50%
Norfloxacin	32–≥64	≥64
Pefloxacin	4–32	16
Ciprofloxacin	1–32	16
Oxacillin	0.12–≥64	4
Vancomycin	0.5–1	1
Gentamicin	2–≥64	≥64
Imipenem	≤0.03–≥64	≤0.03

[a] 50%, MIC for 50% of isolates tested.

DISCUSSION

The efficacy of the fluoroquinolones in selective decontamination for patients at high risk of infection has already been reported (4, 13, 15, 25). All of the studies performed, however, examined one of the different quinolones alone or versus other antibiotics; only one comparative study between quinolones has been reported to date in neutropenic patients (14). In our study we compared the modifications of the microflora of bone marrow transplant patients induced by two fluoroquinolones with different characteristics, norfloxacin and pefloxacin. Our microbiological data, however, showed no relevant difference between the two drugs. No consistent modification of the oral flora was detected with either norfloxacin or pefloxacin. In the intestine both of them, as expected, were highly active against members of the *Enterobacteriaceae* and also against enterococci. The latter effect might be related to the high levels of the drugs in the intestine at the dosage used; in previous studies concentrations in feces of between 440 and 1,900 mg/kg have been reported after 7 days of treatment with norfloxacin (400 mg/day) (5) and of 644 mg/kg after 7 days of administration of pefloxacin (800 mg/day) (10). The high levels in feces could also explain the activity, although moderate, against anaerobes shown in our study, especially by pefloxacin. During treatment we did not observe colonization with potentially pathogenic gram-negative organisms; only one pefloxacin-treated patient was colonized by *C. difficile*.

The most striking effect was the increase in staphylococcal counts: before treatment, patients harbored low counts of staphylococci susceptible to quinolones, after 1 week of treatment, the intestinal aerobic microflora of most patients was replaced with staphylococci resistant to the three quinolones tested. These strains were found to belong to different species, thus excluding the possibility that a single resistant strain had colonized all patients in our hospital. Colonization with resistant staphylococci in the intestine of neutropenic patients treated with the fluoroquinolones has been rarely reported (15). In our study we observed an overgrowth of resistant staphylococci to the same extent with both drugs, despite the reported better in vitro activity of pefloxacin against these organisms. The clinical relevance of our observation needs to be verified: coagulase-negative staphylococci are an increasing cause of infections in neutropenic patients (1, 20). Recently, we found that coagulase-negative staphylococci causing septicemia in neutropenic patients undergoing norfloxacin prophylaxis were resistant to this quinolone (18). It has been suggested that the origin of coagulase-negative staphylococci can also be the gastrointestinal tract (19). In the present study we observed a strain of *S. epidermidis* causing bacteremia with the same pattern of resistance of the strain present in the feces of the patients. The other pathogens isolated during bacteremias in norfloxacin-treated patients were not found in feces or saliva of patients and possibly had their origin from other sites. All of the documented infections occurred in the norfloxacin-treated group; however, the number of patients was too small to allow clinical considerations.

In conclusion, we have shown that both norfloxacin and pefloxacin are good selective decontaminating agents as they effectively eliminate all gram-negative rods from the intestine. No significant difference in the impact on the microflora was detectable between the two regimens.

Further studies are needed to evaluate the frequency and the relevance of the emergence of gram-positive organisms resistant to quinolones in neutropenic patients and whether in the future this may represent a limitation in the widespread use of quinolones as prophylactic agents.

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