Effect of Oral Ofloxacin on Fecal Bacteria in Human Volunteers

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Intestinal members of the family Enterobacteriaceae were eliminated in five human volunteers treated with oral ofloxacin for 5 days. No emergence of resistant Enterobacteriaceae was observed. Counts of group D streptococci were significantly reduced. Colonization by Candida sp. was observed in all five volunteers during ofloxacin treatment. The anaerobic flora was fairly stable from one sample to another before treatment and was not substantially modified by ofloxacin.

The prospective clinical benefits of fluoroquinolones include their uses in severe bacterial gastroenteritis and selective decontamination of the gastrointestinal tracts of neutropenic patients (6). Elimination of fecal aerobic gram-negative bacteria and concomitant stability of the anaerobic flora have been described after ingestion of norfloxacin (5, 8) or ciprofloxacin (3) by human volunteers. It is probably related to the enterohepatic circulation of fluoroquinolones (6). Since another new fluoroquinolone, ofloxacin, is more active in vitro against anaerobic bacteria than are the other fluoroquinolones (9, 10, 12), we thought it of interest to study the effect on fecal bacteria of its administration to human volunteers.

Five healthy, fully informed adults received oral ofloxacin twice daily for 5 days in doses of 200 mg. None had taken antimicrobial agents for at least 1 month before the study. Blood samples were drawn on the first and the last days of treatment at 1 h after drug ingestion. Freshly passed fecal samples were obtained once before treatment, daily during treatment, and daily for 1 week thereafter.

Fecal anaerobes were counted on Araki agar in an anaerobic glove box as described earlier (2). In five volunteers, a total of 814 clones which were recovered from 10⁻⁷ or 10⁻⁹ dilutions of samples obtained before and after 4 days of treatment (82 ± 7 clones per sample) were classified according to their Gram stain properties, shape and positions of spores, and growth characteristics under anaerobic and aerobic conditions, as previously described (8). Anaerobes were classified as oxygen-sensitive or -tolerant strains, based on their resistance to 1 h of exposure to atmospheric oxygen (1).

Group D streptococci were counted on bile-esculin agar (Difco Laboratories, Detroit, Mich.), and staphylococci were counted on mannitol-salt agar (Bio-Mérieux, Charbonnières-les-Bains, France). Organisms of the Enterobacteriaceae family were counted on Drigalski agar (IPP, Paris, France) without antimicrobial agents or supplemented with either 4 µg of ofloxacin (kindly provided by Diamant Pharmaceuticals) per ml or 4 or 40 µg of nalidixic acid (Winthrop Laboratories, Clichy, France) per ml. These media were incubated for 48 h at 37°C. Yeast cells were counted on Sabouraud dextrose agar (Difco) incubated at room temperature for 1 week.

Determination of MICs was by the method of Steers et al. (11) on Mueller-Hinton agar (IPP) for aerobic bacteria and on Araki agar (2) inside the glove box for anaerobes. Control Escherichia coli 7624 was included in each test.

The ofloxacin concentrations in human sera and feces were measured by agar diffusion assay (4). Mueller-Hinton agar at pH 7.3 and Klebsiella pneumoniae 1082E were used. Bacterial counts were converted into common logarithms. Counts of less than 2.0 log₁₀ CFU/g of feces (the minimum detectable concentration) were converted to 2.0 for calculation of some of the mean values given in Table 1.

In serum, the mean peak ofloxacin concentrations (± the standard deviations) rose from 2.8 ± 1.4 µg/ml on day 1 of treatment to 4.2 ± 1.6 µg/ml on day 5 (P < 0.01 by paired Student’s t test). Fecal ofloxacin concentrations were much higher and peaked at 327 ± 274 µg/g of feces after 4 days of treatment. Five days after cessation of therapy, no antibiotic activity was recovered in the feces of any volunteer.

The ofloxacin MICs for 50 and 90% of the 95 clones of Enterobacteriaceae isolated before treatment were low (0.06 and 0.125 µg/ml, respectively). Counts of these Enterobacteriaceae dropped sharply after the beginning of ofloxacin ingestion (Table 1). None were detectable in any volunteer after 4 days of treatment. Six days after the end of ofloxacin administration, Enterobacteriaceae had not yet returned to pretreatment levels (P < 0.05 [Table 1]). No Enterobacteriaceae resistant to nalidixic acid or ofloxacin were isolated from the 45 fecal samples analyzed before, during, or after ofloxacin treatment.

Total counts of anaerobes were not modified by ofloxacin treatment (Table 1). The MICs for 50 and 90% of the anaerobes isolated before (420 clones) or during (394 clones) ofloxacin treatment were equivalent (32 and 128 versus 64 and 128 µg/ml, respectively)). A study of the relative proportions of the various groups of anaerobes present in the predominant fecal flora before and during ofloxacin ingestion showed that no significant changes (paired Student’s t test) were induced by the treatment. Percentages of gram-positive bacilli without visible spores varied from 78 ± 5 to 80 ± 3%, those of gram-positive bacilli with visible spores varied from 8 ± 3 to 9 ± 3%, those of gram-negative bacilli varied from 10 ± 5 to 5 ± 3%, and those of gram-positive cocci varied from 4 ± 2 to 7 ± 3%. These changes were not greater than the variations observed among the volunteers before treatment (data not shown). An increase close to statistical significance (29 ± 17 versus 53 ± 13%; P = 0.065) was observed in the percentages of oxygen-sensitive anaerobes during treatment. It might be due to the greater activity of ofloxacin against the oxygen-tolerant anaerobes isolated before treatment than against the oxygen-sensitive
anaerobes isolated concurrently (MICs for 50% of the isolates, 4 versus 32 μg/ml, respectively).

The ofloxacin MICs for 50 and 90% of the group D streptococci were intermediate before treatment (4 and 8 μg/ml, respectively). Interestingly, the counts of group D streptococci decreased significantly (P < 0.01) during ofloxacin treatment, but bacteria of this group were not completely eliminated (Table 1). Mean counts of staphylococci were low before, during, and after ofloxacin treatment (Table 1).

Yeasts were isolated from the fecal samples of only two of five volunteers before treatment (2.9 and 4.0 log10 CFU/g of feces). However, all five volunteers were colonized by Candida sp. after 4 days of treatment (mean, 3.9 ± 0.6 log10 CFU/g of feces [individual data not shown]).

These results show that ofloxacin treatment induced selective elimination of aerobic gram-negative bacteria in all of the volunteers. Enterobacteriaceae were eliminated, counts of group D streptococci were affected to a lesser extent, and counts of anaerobes remained unchanged. No selection of aerobic gram-negative bacilli resistant to nalidixic acid or ofloxacin was observed. Counts of staphylococci were not modified by ofloxacin treatment. No major qualitative disturbance of the predominant anaerobic flora was induced by ofloxacin. However, colonization of all of the volunteers by Candida sp. during treatment might indicate that ofloxacin induced some disturbance of colonization resistance in the volunteers. A reduction of colonization resistance to C. albicans was also observed previously in human flora-associated gnotobiotic mice during treatment with norfloxacin (8) or erythromycin (1).

The composition of the dominant anaerobic flora of the untreated volunteers was similar to that observed previously (8). This indicates that this flora is fairly stable in humans.

Comparison of MIC determinations with fecal concentrations of ofloxacin showed that ofloxacin was more active in vitro than in the intestinal lumen. Although intestinal concentrations were in the range of several hundred micrograms per gram of feces, only strains with ofloxacin MICs below 1 μg/ml were readily eliminated during treatment (Table 1). Similar differences between in vitro activity and in vivo activity in the intestinal lumen have been previously observed with nifurazide (7) and norfloxacin (8). It might be explained by inactivation of quinolones by feces (13) and may account for the paradoxical failure to detect more alterations in the composition of the anaerobic flora.

In conclusion, the results of this study showed that, despite its in vitro activity against anaerobes, the effect of oral ofloxacin on fecal bacteria in human volunteers is comparable to that of the other fluoroquinolone derivatives.

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LITERATURE CITED


