THE EFFECT OF LEVOFLOXACIN, AN OPTICALLY-ACTIVE ISOMER OF OFLOXACIN, ON FECAL MICROFLORA IN HUMAN VOLUNTEERS

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Following oral administration of levofloxacin (LVFX, (S)-(−)-Ofloxacin; formerly designated as DR-3355) at 200 mg per dose 3 times a day for 7 days to 6 healthy male volunteers, degrees of disturbance of the fecal microflora and fecal drug concentrations were examined.

The total viable count remained unchanged during the study period due to the minimal change in the number of members of the family Bacteroidaceae, the most predominant organisms. Most of the aerobes including facultative anaerobes were suppressed by LVFX with only a slight increase in yeasts. In particular, the members of the family Enterobacteriaceae were reduced to below the detection limit on and after day 3 through the time of discontinuation of the drug in all subjects but one. Among the obligate anaerobes, peptostreptococci and bifidobacteria decreased or disappeared in some volunteers, but no significant changes were observed in other anaerobes. Neither Clostridium difficile nor its toxin D-1 was detected in any of the volunteers. No side effects attributable to the drug were observed.

During administration, LVFX was detected in the feces at high concentrations which correlated well with the decrease of susceptible members of flora as well as to their detection rate.

During the past decade, several new quinolone antibacterial agents have been introduced into clinical use. Among them, ofloxacin (OFLX) is characterized by its broad and high antibacterial activity over common Gram-positive and Gram-negative aerobic pathogens and obligate anaerobes1), as well as high oral absorption2). When administered orally, OFLX caused a drastic decrease in the number of members of the family Enterobacteriaceae, which were highly susceptible to the drug, but did not cause any significant decrease in the total count of the intestinal bacteria in normal subjects3). Selective overgrowth or superinfection of Clostridium difficile was not observed3).

The present drug levofloxacin (LVFX), formerly designated as DR-3355, an optical isomer of OFLX, i.e., (S)-(−)-OFLX (Fig. 1), has twice the potency of OFLX4—7) while demonstrating the same good oral absorption in humans as OFLX8). In the present study, we examined the effect of LVFX on the intestinal

Fig. 1. Chemical structure of LVFX, (−)-(S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid hemihydrate.
Subjects and Methods

Subjects

The subjects enrolled in the study were 6 healthy adult male volunteers. The subjects signed a form of informed consent after being fully appraised of the purpose and contents of the study in detail. Their mean age was 36.8 ± 7.2 years (mean ± standard deviation) with an age range of from 26 to 44 years. The mean body weight was 69.0 ± 6.0 kg (range, 61 ~ 76.2 kg). The study criteria specifically excluded subjects suffering from any chronic disease or allergy to drugs, and those who had taken any antibiotics including LVFX for a month before initiation of the present study: all 6 volunteers satisfied the criteria for inclusion in this study.

Dosage

Two tablets, each containing 100 mg of LVFX, were orally administered 3 times a day after meals on 7 consecutive days (Fig. 2).

Safety evaluation for volunteers

The following tests were carried out before starting the study and on day 7: whole blood cell counts, blood biochemistry and urinalysis, and physiological function tests including electroencephalography.

Collection of feces

Approximately 1 g of feces, freshly voided, was placed in 9 ml of a sample transport medium, brain heart infusion broth (Difco Laboratories, Detroit, Mich.), consisting of 0.1% agar, 0.05% L-cysteine-HCl·H2O, 0.4% Na2CO3, pH 7.4 adjusted with oxygen-free CO2, and was kept at 4°C until processed. The time sequence of administration of LVFX and collection of fecal specimens are shown in Fig. 2.

Examination of fecal microflora

In the present study, we did not intend to make an identification of the organisms grown on plating media at species level, but only to classify them at the level of family, genus, or group, in order to follow the degree and time course of any disturbance of the intestinal microflora by the administration of LVFX. For this purpose, bacteriological analysis of the fecal microflora was carried out according to the methods described by MITSUOKA et al.9-11). Fecal specimens in the transport media were homogenized under an anaerobic atmosphere by blowing oxygen-free CO2 gas into the sample tubes, giving a tenfold dilution of feces. These suspensions were then further diluted to make hundredfold serial dilutions of 10⁻¹, 10⁻³, and 10⁻⁷ with a buffer solution (0.45% KH2PO4, 0.6% Na2HPO4, 0.1% agar, 0.05% L-cysteine-HCl·H2O and 0.05% Tween 80, pH 7.0). To make differential counts of aerobes, a portion of 0.05 ml of each dilution including 10⁻¹ was spread on a one-fourth section of each agar plate containing the following selective and non-selective media: (non-selective medium) Trypticase Soy (TS) agar (BBL Microbiology Systems,
Cockeysville, Md.) containing 5% defibrinated horse blood; and the following selective media: triphenyltetrazolium chloride-acridine orange-thallous sulfate-esculin-crystal violet (TATAC) agar for enterococci; deoxycholate-hydrogen sulfide-lactose (DHL) agar (Eiken Chemical, Tokyo) for Enterobacteriaceae; phenylalcohol-egg yolk suspension (PEES) agar for staphylococci; and potato dextrose (PD) agar (Difco) for yeasts. For differential counts of anaerobes, the plating media used were as follows: neomycin-brilliant green-taurocholate-blood (NBGT) agar for Bacteroidaceae, bifidobacteria-selective (BS) agar for bifidobacteria; neomycin-Nagler (NN) agar for clostridia; eubacteria-selective (ES) agar for eubacteria; modified veillonella-selective (VS) agar for veillonellae; and modified lactobacilli-selective (LBS) agar for lactobacilli. Further 10⁻⁸ dilution of the fecal suspension was made to inoculate non-selective agar plates for anaerobic organisms, i.e., modified Eggerth-Gagnon (EG) agar and glucose-liver-blood (BL) agar. A quantity of 0.05 ml of 10⁻⁵, 10⁻⁷, and 10⁻⁸ dilutions of each specimen was spread on a one-third section of these plates. Five agar plates (TS, DHL, TATAC, PD, and PEES agars) for detection of aerobes were incubated aerobically at 37°C for 24 to 48 hours and 8 agar plates (EG, BL, BS, NBGT, ES, NN, VS, and LBS agars) for detection of anaerobes were incubated at 37°C for 48 to 96 hours in an anaerobic glove box (Anaerobic system model 1024, Forma Scientific Laboratories, Marietta,
Fig. 4. Effects of LVFX on fecal microflora of volunteer No. 2 and concentrations of the drug in the fecal specimens.

Symbols: Total viable count ○——○. Viable counts of anaerobes

Bacteroidaceae □—□, Eubacteria ●——●, Bifidobacteria △—△, Peptostreptococci ●——●, Clostridia (Lecithinase-positive) △—△, Clostridia (Lecithinase-negative) △—△, Veillonella ○——○.

Viable counts of aerobes

Lactobacilli △—△, Enterococci ●——●, Enterobacteriaceae ○——○, Staphylococci □——□, Bacilli △—△, Yeast ●——●.

Ohio) under a gas mixture of 80% N₂, 10% H₂, and 10% CO₂. After incubation, different colony types were counted. Colonies from the anaerobic agar plates were subcultured on BL agar plate to aerobic incubation. No growths of the subcultured organisms under aerobic condition were identified as strictly anaerobic bacteria. All of the organisms isolated were identified by means of morphological tests as indicated by MITSUOKA et al.⁹⁻¹¹, and were classified into appropriate bacterial groups. The number of each organism was calculated, and expressed as a logarithmic number. The lower limit of detection was set at 2.3/g of feces.

Detection of C. difficile

Fecal specimens used for the examination of microflora as described above were also plated on cycloserine-cefoxitin-fructose agar medium containing 0.1% sodium taurocholate¹² and the plates were incubated under anaerobic condition. The plates were pretreated in an anaerobic glove box before use. Detection of toxin D-1 was performed using the Latex agglutination kit (Shionogi & Co., Osaka) which utilizes a specific antibody against the toxin. The lowest detection limit of the toxin in the transported fecal specimen was set at 5 μg/ml.
Detection of LVFX-resistant bacteria
To detect LVFX-resistant organisms of the families Enterobacteriaceae and Bacteroidaceae, DHL agar plates containing 12.5 μg/ml of LVFX and NBGT agar plates containing 100 μg/ml of LVFX, respectively, were inoculated with the fecal dilutions. Colonies grown on these plates were considered as LVFX-resistant organisms. The resistant Bacteroides spp. thus obtained were identified by Minitek Anaerobe (BBL).

Determination of concentration of LVFX in fecal specimens
The concentration of LVFX in each specimen was determined by a bioassay using Escherichia coli Kp or Bacillus subtilis ATCC 6051 as indicator organisms, as previously described for OFLX. The lower limit of assay was set at 1.9 μg/g of feces.

Results
Effects of LVFX on fecal microflora
A decrease in the total viable count was observed in volunteer No. 3 on day 3 (Fig. 5), while that in other volunteers was almost constant with minor fluctuations (Figs. 3, 4, 6, 7, 8, and 9).
Fig. 6. Effects of LVFX on fecal microflora of volunteer No. 4 and concentrations of the drug in the fecal specimens.

Symbols: Total viable count ○—○. 
Viable counts of anaerobes
Bacteroidaceae ▲—▲, Eubacteria ●—●, Bifidobacteria △—△, Clostridia (Lecithinase-positive) ▲—▲, Clostridia (Lecithinase-negative) ▽—▽, Veillonella ○—○.
Viable counts of aerobes
Lactobacilli △—△, Enterococci ●—●, Enterobacteriaceae ○—○, Staphylococci □—□, Bacilli ▲—▲, Yeast ●—●.

Among the obligate anaerobes, the number of members of the family Bacteroidaceae, the most predominant members in the fecal flora, showed a similar change to the total viable count. As for the other anaerobic flora, the viable counts of all bacterial groups in volunteer No. 3 decreased on day 3. Particularly, bifidobacteria were eliminated during the period of administration of the drug. In other volunteers no significant decrease in the count of bifidobacteria was observed, except a slight decrease in volunteers Nos. 1 and 2 during LVFX administration. Peptostreptococci were below the detection limit in volunteer No. 4, whereas in the others the organisms showed a tendency to decrease during administration of the drug, and in volunteers Nos. 5 and 6, they were eliminated completely from day 3 through 8 (Figs. 7 and 8). Lecithinase-negative clostridia [clostridia (−)] showed a tendency to decrease in volunteers Nos. 1, 2, 5, and 6, while the organisms increased in volunteer No. 4. Lecithinase-positive clostridia[clostridia (+)], minor organisms among the anaerobic microflora, tended to decrease during the administration period. Veillonella decreased in volunteers Nos. 1, 5 and 6. Averaging the data from all 6 subjects (Fig. 9), a tendency towards a decrease in the numbers of bifidobacteria, peptostreptococci, clostridia (+) and Veillonella was noticed during administration of the drug. A significant simplification in the morphology of the colonies grown on the plates was observed in each fecal specimen of the volunteers during the administration period.
As for the changes in the facultative anaerobes and aerobes of the microflora, members of the family Enterobacteriaceae drastically decreased in all volunteers right after administration of the drug except volunteer No. 6. The organisms in volunteer No. 6 gradually decreased and disappeared on day 3 during administration of the drug; even by day 21, the organisms had not reappeared. Enterococci also showed a tendency of decrease, especially in volunteer No. 3 in whom the organisms decreased markedly during administration of the drug. The effects on lactobacilli and bacilli varied depending on the subjects. Yeasts showed signs of an increase in some volunteers, and significant particularly in volunteers Nos. 3, 4, and 6. In volunteer No. 3, yeasts became the most predominant among the aerobic microflora.

Detection frequency of each group of organisms in all 6 volunteers during the course of experiment (Table 1) coincided with the changes in the number of susceptible members of the fecal flora. In other words, during administration of the drug, bifidobacteria, peptostreptococci, the Enterobacteriaceae, clostridia (+), and bacilli decreased, while yeasts showed a slight outgrowth.

Signs of recovery from the effect of the drug on fecal microflora was observed at 14 days after the end of administration of the drug, but recovery was by then still incomplete.
Examination of *C. difficile*

No toxin was detectable up to and including 7 days after the conclusion of LVFX administration. Only organisms with a different morphology from that of *C. difficile* could be detected by Gram staining.

Detection of LVFX-resistant bacteria

None of the strains of members of the family Enterobacteriaceae could be observed which were resistant to LVFX at a concentration of 12.5 μg/ml. On the other hand, in all volunteers except volunteers Nos. 3 and 4, proportions of the number of LVFX-resistant organisms of the family Bacteroidaceae, at a concentration of 100 μg/ml, showed a tendency to increase during the administration period against the total number of Bacteroidaceae grown on NBGT agar plates containing no drug (Table 2). However, the proportion decreased after the treatment, except volunteer No. 4 who had already been shown to harbor resistant organisms on day 0. Resistant organisms identified were mostly *Bacteroides vulgatus* in the volunteers Nos. 1, 3, 4, 5, and 6 throughout the experimental period, whereas *Bacteroides ovatus* was detected in volunteer No. 2.
Drug concentrations in the feces

During the period of administration, LVFX was detected in the feces of all 6 volunteers (Figs. 3, 4, 5, 6, 7, and 8). The mean drug concentrations (mean ± standard deviation) were 91.3 ± 45.3 µg/g (range, 52.4 ~ 162.8 µg/g) on day 3, 79.0 ± 25.1 µg/g (range, 41.4 ~ 113.4 µg/g) at 1 day after the end of administration (day 8), and 1.9 ± 4.7 µg/g (range, <1.9 ~ 11.5 µg/g) at 4 days after termination of administration (day 11) (Fig. 9). The time course change of fecal drug concentration was inversely related to detection of all aerobes except yeasts, and anaerobes, especially peptostreptococci and clostridia (+). On the other hand, yeast counts demonstrated a parallel relationship to the fecal concentrations of the drug. The detected drug concentrations also coincided with the frequency of detection in each group of the organisms (Table 1).

Reaction to drug administration

No obvious side effects, such as diarrhoea, were observed in any of the volunteers throughout the study, although volunteers Nos. 4, 5, and 6 experienced an increase in the frequency of evacuation of feces. No
Table 1. Detection frequencies of intestinal microflora in 6 volunteers orally administered with LVFX.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Percentage of positive case on day*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Bacteroidaceae</td>
<td>100</td>
</tr>
<tr>
<td>Eubacteria</td>
<td>100</td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>100</td>
</tr>
<tr>
<td>Peptostreptococci</td>
<td>83.3</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>100</td>
</tr>
<tr>
<td>Enterococci</td>
<td>100</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>100</td>
</tr>
<tr>
<td>Clostridia (Lec')</td>
<td>83.3</td>
</tr>
<tr>
<td>Clostridia (Others)</td>
<td>100</td>
</tr>
<tr>
<td>Veillonella</td>
<td>83.3</td>
</tr>
<tr>
<td>Staphylococci</td>
<td>0</td>
</tr>
<tr>
<td>Bacilli</td>
<td>50</td>
</tr>
<tr>
<td>Yeast</td>
<td>66.7</td>
</tr>
</tbody>
</table>

* Day of study: See Fig. 2. b: Lecithinase-positive.

Table 2. Proportion of LVFX-resistant Bacteroidaceae in fecal flora of healthy adult volunteers.

<table>
<thead>
<tr>
<th>Volunteer No.</th>
<th>0</th>
<th>3</th>
<th>8</th>
<th>11</th>
<th>14</th>
<th>21</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>5.3*</td>
<td>12</td>
<td>16</td>
<td>0.2</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>9.2</td>
<td>1.7</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>3</td>
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<td>0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>43</td>
<td>25</td>
<td>18</td>
<td>74</td>
<td>43</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>1.3</td>
<td>3.4</td>
<td>2.2</td>
<td>2.4</td>
<td>0.1</td>
</tr>
<tr>
<td>6</td>
<td>0.7</td>
<td>32</td>
<td>42</td>
<td>19</td>
<td>16</td>
<td>2.6</td>
</tr>
</tbody>
</table>

* Percent of LVFX-resistant Bacteroidaceae in total Bacteroidaceae.

abnormality in the volunteers was observed with respect to blood cell counts, blood biochemistry and urinalysis, and physiological functions.

**Discussion**

The drug in the present study, LVFX, is known in in vitro susceptibility tests to have an excellent antimicrobial activity not only against Gram-positive and Gram-negative aerobes but also against *Bacteroides fragilis*. However, changes in the fecal microflora in humans following continuous oral administration of the drug have confirmed that the drug had a slight growth-suppressive effect against some members of anaerobic bacteria, whereas, in the aerobic microorganisms, it revealed strong antibacterial effects against Enterobacteriaceae and enterococci.

In spite of detection of high concentrations of the drug in the feces of each individual, there was little change in the total viable counts of the fecal microflora. This is accounted for by the constant numbers of the most predominant organisms, i.e., members of the family Bacteroidaceae. One of the reasons for the minor changes in the number of these organisms after administration of LVFX could be due to an increase of organisms resistant to the drug. This is supported by the actual increase of LVFX-resistant Bacteroidaceae which replaced susceptible members in all but 2 volunteers. Among anaerobic bacteria, peptostreptococci and bifidobacteria decreased or disappeared in some volunteers following administration of LVFX; an additional tendency to decrease was seen in *Veillonella* and clostridia (+), minority groups of anaerobes in the fecal flora. The degree of this change in the latter organisms was little affected even when the fecal concentration of the drug increased. Judging from the less-diversified morphology of colonies appearing on the agar plates, it may be speculated that certain types of LVFX-resistant bacteria increased,
and replaced susceptible counterparts, with the result that the apparent composition of the fecal microflora was not noticeably changed.

Among facultative anaerobic and aerobic bacteria, the Enterobacteriaceae and enterococci tended to decrease during and after administration of the drug. In particular, members of the family Enterobacteriaceae drastically decreased soon after administration of the drug, eventually disappearing below the detection limit. Yeasts tended to increase in some volunteers while they decreased in others. In volunteer No. 3, yeasts were the predominant organism in the aerobic microorganisms on day 3 whereas the number of other organisms including anaerobic bacteria decreased. However, they disappeared with increased fecal concentration of the drug.

As described in the present paper, the changes in the fecal microflora following administration of LVFX were similar to those caused by OFLX reported previously by us\(^3\) and others\(^13,14\). Since the present drug is an antimicrobially-active isomer of OFLX, i.e., \((S)-(\sim)-\)OFLX, a certain amount of the present drug is known to be equivalent to double the amount of OFLX. This increased dose-effect, however, was only observed as a decrease in the number of peptostreptococci in the present study.

Similar results to those in the present study were obtained with the drugs of a similar group such as enoxacin\(^15\), norfloxacin\(^15\), ciprofloxacin\(^16,17\), pipemidic acid\(^18\) and lomefoxacin, formerly designated as NY-198\(^19\). Considering the major ecological impact of the quinolones on the intestinal microflora, it is very interesting that the effect of the new quinolones, including the present drug, is unique in its marked selective reduction in members of the family Enterobacteriaceae and its negligible effect on predominant anaerobes, different from the effect of the cephem antibiotics\(^18,20\sim25\).

Recovery from the effect of the drug on fecal microflora was not complete in all volunteers even at 14 days after termination of administration of the drug. A further 1 to 2 weeks seems to be required for the complete recovery, based on our previous observations with OFLX\(^3\).

Following administration of LVFX, C. difficile and its toxin D-1 were not detected. No notable side effect was observed following administration of the drug except an increased frequency of evacuation of feces in some volunteers. In this study, it was observed that an overgrowth of yeasts took place in the fecal microflora of some volunteers by administration of the drugs, similar to that in the OFLX trial\(^3\).

It should be emphasized that LVFX is unique in its strong activity to inhibit selectively members of the family Enterobacteriaceae. We also confirmed previously that the drug was highly active against bacterial pathogens associated with travellers' diarrhoea including enteropathogenic E. coli, Salmonella spp. and Shigella spp\(^20\). Therefore the drug could be effective for treatment of gastrointestinal infections with the pathogenic Enterobacteriaceae. A possible additional advantage is seen with the smaller dosage of LVFX needed compared with OFLX.

Acknowledgment

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References