The impact of rufloxacin given as prophylaxis to patients with cancer on their oral and faecal microflora


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A single dose of 200 mg/day rufloxacin was investigated for preventing infection and for its impact on the commensal flora in a pilot study of 62 patients undergoing cytotoxic treatment for cancer. No infection caused by Gram-negative bacilli occurred among 54 assessable patients but prophylaxis was replaced by empirical treatment for fever in 19 cases and because of an adverse event, in a further three cases. The remaining 32 patients completed prophylaxis. The number of oral Branhamella spp., faecal Enterobacteriaceae and Bacteroides spp. were significantly reduced whereas there was little effect of rufloxacin on the numbers of the other oral and faecal microflora. However, resistance to rufloxacin increased among both oral viridans streptococci, coagulase negative staphylococci and the faecal enterococci. These preliminary data suggest that selective oral antimicrobial prophylaxis for patients with cancer might be achieved with once-daily rufloxacin.

Introduction

Several studies have shown that prophylaxis with fluoroquinolones is effective in preventing infections caused by Gram-negative bacilli in neutropenic patients (Dekker, Rosenberg-Arksa, Verhoef, 1987; GIMEMA, 1991; Donnelly et al., 1992). Our experience has shown that ciprofloxacin was more reliable than either ofloxacin or pefloxacin in preventing bacteraemia due to Gram-negative bacilli and also led to fewer instances of colonisation with these bacteria (D’Antonio et al., 1994). However, the drug has to be given twice daily which can sometimes prove difficult when patients are suffering from severe mucositis or nausea. A drug that can be given once-daily may be better tolerated.

The new fluoroquinolone rufloxacin appears to be a suitable candidate for once-daily prophylaxis as it has a long half-life and is more concentrated in the tissues than in plasma giving it greater antibacterial activity in vivo than might be anticipated from in-vitro data (Ravizzola et al., 1992). The quinolone has a similar broad spectrum of
activity to that of ciprofloxacin (Imbimbo et al., 1991; Mattina et al., 1991) and 200 mg/day results in serum concentrations that exceed the MICs for several pathogens (Cocuzza & Williams, 1991). Before attempting a large clinical trial of prophylaxis in patients undergoing chemotherapy for cancer, we decided to perform a pilot study to determine the efficacy of rufloxacin in preventing infection caused by Gram-negative bacilli and its impact on the oral and faecal flora.

**Patients and methods**

**Patients**

Patients 14 years and older undergoing treatment for haematological malignancy or solid tumors likely to reduce the granulocytes to less than \( \leq 0.5 \times 10^9/L \) for at least 8 days were eligible to enter the study providing that their temperature had not exceeded 38°C and they had not received any antimicrobial treatment during the previous 5 days. Patients with any psychiatric or neurological disorder were excluded as were those with impaired renal or liver function, since some chemotherapy regimens lead to temporary impairment of hepatic function.

**Prophylaxis**

Once informed consent was obtained, patients were given 200 mg rufloxacin, 1–3 days before starting intensive cytotoxic chemotherapy. Prophylaxis was continued once daily until the peripheral granulocyte count returned to \( \geq 0.5 \times 10^9/L \) unless either a toxic or allergic reaction occurred or empirical treatment with 2 g ceftazidime tid and 20 mg/kg amikacin od was started for fever. All patients were given tablets of 150 mg fluconazole once daily to prevent fungal infection and none was given colony-stimulating factors. Oral medication was taken under supervision to ensure compliance.

**Surveillance specimens**

A specimen of faeces and a 10 mL saline mouthwash were obtained before starting prophylaxis, once during the next two weeks and again on the last day of treatment. Faeces were suspended in 10 mL saline before further processing. Further samples were taken on day 6 (week 1), day 12 (week 2) and at the end of prophylaxis.

**Isolation media and conditions**

All media were obtained from Biolife Italiana, Milan, Italy unless stated otherwise and each sample was cultured quantitatively. Columbia blood agar containing vitamin K and haemin was used for total aerobic and anaerobic counts. MacConkey agar was employed for Enterobacteriaceae, mannitol salt agar for staphylococci, haematin agar for Gram-negative cocci, pseudomonas selective agar containing nalidixic acid for *Pseudomonas* spp., Mitis-salivarius agar (Difco Laboratories, Detroit, MI, USA) for streptococci and Enterococcosel agar (Becton Dickinson Diagnostic Instrument Systems, Cockeysville, MD, USA) for the enterococci. Each of these media was incubated for 24 h aerobically at 37°C.
Kanamycin-vancomycin blood agar was employed for Bacteroides spp. and Prevotella spp., neomycin-vancomycin blood agar for fusobacteria, Clostridium difficile selective medium and egg-yolk neomycin agar (Unipath Oxoid, Basingstoke, UK) for other Clostridium spp. and each were incubated for 48 h anaerobically at 37°C.

Identification and susceptibility tests
Isolates were identified using standard criteria (Finegold & Baron, 1986). The antimicrobial susceptibility of the Enterobacteriaceae, viridans streptococci, staphylococci and enterococci was determined by broth microdilution according to the procedure recommended by the American National Committee for Clinical Laboratory Standards (NCCLS, 1990) adopting a breakpoint of ≤8 mg/L rufloxacin (European Study Group on Antibiotic Breakpoints, 1993).

Definitions
A single isolation of a given organism was considered to represent transient carriage whereas isolation on two or more consecutive occasions was regarded as a colonisation. To assess the impact of prophylaxis on the microflora each patient had to have complied with treatment for at least 10 days.

Other investigations
Patients were examined daily for possible sites of infection such as mucositis, cellulitis, perineal lesions and others and appropriate specimens were obtained for culture where possible. Blood was obtained for culture when fever developed.

Statistical analysis
Microbial counts and MICs were transformed to log_{10} and log_{2} respectively and data were analysed using Systat 5 (Systat Inc., Evanston, IL, USA). The analysis of variance was employed to test whether differences in microbial numbers found during treatment were significant at the 5% level and a non-linear regression model was used to analyse changes in the MICs.

Results
Sixty-two patients were originally entered into the study between September 1993 and May 1994. The treatment episodes of five patients were excluded from analysis as the duration of granulocytopenia was less than 8 days. A further three patients were treated with intravenous antimicrobial agents for fever at the time of entering the study.

Outcome of prophylaxis
Compliance was judged to be adequate, with >90% intake of the prescribed tablets. Prophylaxis was discontinued in three cases because two patients developed a skin rash and a third patient experienced gastrointestinal disturbance. Empirical therapy was given to 19 patients who developed fever after a mean of 6.3 ± 1.8 days therapy. Five of these patients had a clinically defined infection and six patients had a
microbiologically defined infection of which four had bacteraemia due to viridans streptococci (three *Streptococcus mitis*, one *Streptococcus sanguis*), one had a genitourinary tract infection due to *Staphylococcus haemolyticus* and another skin soft tissue infection due to *Staphylococcus xylosus*. The impact of prophylaxis on the microflora could be assessed in the 32 patients who continued prophylaxis treatment until their granulocytes recovered. Their characteristics are detailed in Table I.

**Impact on Enterobacteriaceae**

The oral cavity of one patient was colonised with *Escherichia coli* and that of another with *Klebsiella oxytoca* and transient carriage with *Enterobacter cloacae* and *Enterobacter agglomerans*, respectively, was detected in another two cases. Two other patients showed faecal colonisation with *Pseudomonas aeruginosa* and a further one with *Klebsiella oxytoca*. Each organism was isolated in numbers of less than $10^5$ cfu/mL but each was resistant to $>32$ mg/L rufloxacin.

**Impact on the viable counts of the oral microflora**

There was a significant reduction in *Branhamella* spp. which had fallen from a mean of 4.2 to 1.8 cfu/mL within the first week of prophylaxis with samples from 18 patients (65%) showing a $>\log 10$ reduction. However, there was a significant decrease in the numbers of viridans streptococci, coagulase-negative staphylococci or anaerobic bacteria (Table II).

**Impact on the viable counts of the faecal microflora**

By the end of the first week of treatment, there was a significant decrease in the mean number of *Bacteroides* spp. and Enterobacteriaceae in faecal samples but little further change thereafter (Table III). A $>\log 10$ reduction in the Enterobacteriaceae was observed and in the samples of all patients and *Bacteroides* spp. in 25% of patients. There was no significant change in the numbers of anaerobic cocci, coagulase negative staphylococci, enterococci or *Clostridium* spp.

**Impact on susceptibility of oral microflora**

There was a significant increase in the resistance of both the viridans streptococci and coagulase negative staphylococci to rufloxacin which was best described by a non linear quadratic regression model with mean $\log_2$ MICs for both increasing significantly during the first two weeks then remaining virtually unchanged thereafter (Table IV).

### Table I. Patients' characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>32</td>
</tr>
<tr>
<td>Males/females</td>
<td>15/17</td>
</tr>
<tr>
<td>Median age</td>
<td>61 (25-75)</td>
</tr>
<tr>
<td>Underlying disease</td>
<td></td>
</tr>
<tr>
<td>lymphomas</td>
<td>14</td>
</tr>
<tr>
<td>solid tumours</td>
<td>18</td>
</tr>
<tr>
<td>Median duration of prophylaxis days (range)</td>
<td>16 (11-35)</td>
</tr>
</tbody>
</table>
### Table II. Impact of rufloxacin prophylaxis on oral microbial flora

<table>
<thead>
<tr>
<th>Species</th>
<th>Before mean ± S.D.</th>
<th>1 week prophylaxis mean ± S.D.</th>
<th>2 weeks' prophylaxis mean ± S.D.</th>
<th>Last day of prophylaxis mean ± S.D.</th>
<th>Significance (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobic cocci (log_{10} cfu/mL)</td>
<td>3.0 ± 1.1</td>
<td>2.6 ± 1.2</td>
<td>2.6 ± 1.4</td>
<td>2.8 ± 0.9</td>
<td>P = 0.4333</td>
</tr>
<tr>
<td>Bacteroides (log_{10} cfu/mL)</td>
<td>2.8 ± 1.4</td>
<td>2.8 ± 1.6</td>
<td>2.9 ± 1.4</td>
<td>3.2 ± 1.4</td>
<td>P = 0.5363</td>
</tr>
<tr>
<td>Branhamella (log_{10} cfu/mL)</td>
<td>4.2 ± 1.4</td>
<td>1.8 ± 1.2</td>
<td>1.4 ± 1.1</td>
<td>1.3 ± 1.1</td>
<td>P = 0.0000</td>
</tr>
<tr>
<td>Fusobacteria (log_{10} cfu/mL)</td>
<td>1.8 ± 1.2</td>
<td>1.7 ± 1.2</td>
<td>1.9 ± 1.2</td>
<td>1.9 ± 1.2</td>
<td>P = 0.8857</td>
</tr>
<tr>
<td>Staphylococci (log_{10} cfu/mL)</td>
<td>2.3 ± 0.8</td>
<td>2.5 ± 0.8</td>
<td>2.5 ± 1.0</td>
<td>2.3 ± 0.8</td>
<td>P = 0.8080</td>
</tr>
<tr>
<td>Viridans streptococci (log_{10} cfu/mL)</td>
<td>5.7 ± 0.8</td>
<td>5.7 ± 0.7</td>
<td>5.5 ± 0.6</td>
<td>5.7 ± 0.6</td>
<td>P = 0.4057</td>
</tr>
</tbody>
</table>

### Table III. Resistance induced by rufloxacin on oral staphylococci and viridans streptococci

<table>
<thead>
<tr>
<th>Species</th>
<th>Before mean ± S.D.</th>
<th>1 week prophylaxis mean ± S.D.</th>
<th>2 weeks' prophylaxis mean ± S.D.</th>
<th>Last day of prophylaxis mean ± S.D.</th>
<th>Significance (quadratic regression)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococci log_{10} MIC (mg/L)</td>
<td>1.8 ± 2.2</td>
<td>2.8 ± 2.2</td>
<td>3.0 ± 2.2</td>
<td>3.0 ± 2.2</td>
<td>1.62 -0.25</td>
</tr>
<tr>
<td>Viridans streptococci log_{10} MIC (mg/L)</td>
<td>2.8 ± 1.7</td>
<td>3.6 ± 1.0</td>
<td>4.0 ± 0.6</td>
<td>4.1 ± 0.7</td>
<td>1.26 -0.17</td>
</tr>
</tbody>
</table>

*Regression coefficients.
Impact on susceptibility of faecal microflora

The mean log$_2$ MIC of rufloxacin for faecal enterococci also significantly increased from 1.8 to 3.3 by the second week of treatment (Table V) whereas the susceptibility of the faecal coagulase negative staphylococci and the other bacteria did not change during treatment.

Discussion

Prophylaxis with rufloxacin successfully suppressed infection due to Gram-negative bacilli as have the other fluoroquinolones we have used in the past (D'Antonio et al., 1994). These results are encouraging as this was achieved using only 200 mg/day rufloxacin whereas other fluoroquinolones are given at higher doses twice daily. However, oral colonisation with Enterobacteriaceae resistant to >32 mg/L rufloxacin occurred in two cases and another three patients showed faecal colonisation, two with *P. aeruginosa* and one with *K. oxytoca*. Although none developed infection, these data indicate that there is a potential risk of patients becoming colonised by resistant Gram-negative bacilli during prophylaxis with rufloxacin which may lead to infections with these bacteria as has occurred after treatment with pefloxacin and ofloxacin (Cometta et al., 1994; Kern et al., 1994).

Prophylaxis was successful in 32 (59%) of cases who remained free of infection during neutropenia but had to be discontinued in three cases because of adverse reactions and, in another 19 cases because fever developed. The results are in line with those we obtained using other fluoroquinolones (D'Antonio et al., 1994) though somewhat lower than those reported in other studies (Dekker et al., 1987; Donnelly et al., 1992; The GIMEMA infection program, 1991; Jansen et al., 1994).

Suppression of the Gram-negative bacilli in the oral cavity and intestines without undue disturbance of the other commensal flora is a precondition for successful selective antimicrobial prophylaxis (Donnelly, 1993a). Among the oral microflora, only *Branhamella* spp. decreased in numbers within the first week of prophylaxis as did the numbers of faecal Enterobacteriaceae. The other microflora were unaffected but the numbers of faecal *Bacteroides* spp. declined significantly in 25% of the cases. This might have been caused by an increase in the oxygen concentration in the gut as a result of the reduction in faecal aerobes (Edlund et al., 1988). This effect upon these anaerobes appears to be a characteristic feature of the newer fluoroquinolones and has been noted after a single dose of 400 mg rufloxacin (Marco et al., 1995).

Resistance to rufloxacin among the viridans streptococci and coagulase negative staphylococci increased during the first two weeks of prophylaxis as has been noted during prophylaxis with ciprofloxacin (Donnelly, 1993b). Thus, by the time neutropenia occurs, these organisms are already resistant. Only four (6%) of the patients in our study developed bacteraemia due to viridans streptococci but this number would have been higher had they received cytoreductive treatment likely to induce severe oral mucositis as this appears to exert a greater influence than does prophylaxis (Donnelly, 1995).

Our preliminary data indicate that rufloxacin may offer a simpler prophylactic regimen for patients receiving chemotherapy for cancer. However, this can only be confirmed by undertaking formal clinical trials that pay particular attention to the
Table IV. Impact of rufloxacin prophylaxis on faecal microbial flora

<table>
<thead>
<tr>
<th>Species</th>
<th>Before mean ± S.D.</th>
<th>1 week prophylaxis mean ± S.D.</th>
<th>2 weeks' prophylaxis mean ± S.D.</th>
<th>Last day of prophylaxis mean ± S.D.</th>
<th>Significance (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobic cocci (log10 cfu/g)</td>
<td>3.8 ± 1.9</td>
<td>3.2 ± 2.1</td>
<td>3.5 ± 1.9</td>
<td>3.9 ± 1.7</td>
<td>P = 0.3859</td>
</tr>
<tr>
<td>Bacteroides (log10 cfu/g)</td>
<td>9.5 ± 0.7</td>
<td>8.9 ± 1.2</td>
<td>8.6 ± 1.1</td>
<td>8.7 ± 1.1</td>
<td>P = 0.0038</td>
</tr>
<tr>
<td>Clostridia (log10 cfu/g)</td>
<td>4.6 ± 1.0</td>
<td>4.2 ± 1.5</td>
<td>4.5 ± 1.1</td>
<td>4.6 ± 1.0</td>
<td>P = 0.3870</td>
</tr>
<tr>
<td>Enterobacteriaceae (log10 cfu/g)</td>
<td>7.8 ± 0.9</td>
<td>2.0 ± 1.5</td>
<td>1.5 ± 1.4</td>
<td>1.4 ± 1.4</td>
<td>P = 0.0000</td>
</tr>
<tr>
<td>Enterococci (log10 cfu/g)</td>
<td>5.0 ± 1.3</td>
<td>4.9 ± 0.9</td>
<td>5.2 ± 0.7</td>
<td>5.2 ± 0.7</td>
<td>P = 0.9451</td>
</tr>
<tr>
<td>Staphylococci (log10 cfu/g)</td>
<td>1.6 ± 1.6</td>
<td>1.6 ± 1.6</td>
<td>1.6 ± 1.6</td>
<td>1.9 ± 1.7</td>
<td>P = 0.8631</td>
</tr>
</tbody>
</table>

Table V. Resistance induced by rufloxacin on faecal staphylococci and enterococci

<table>
<thead>
<tr>
<th>Species</th>
<th>Before mean ± S.D.</th>
<th>1 week prophylaxis mean ± S.D.</th>
<th>2 weeks' prophylaxis mean ± S.D.</th>
<th>Last day of prophylaxis mean ± S.D.</th>
<th>β₁</th>
<th>β₂</th>
<th>Significance (quadratic regression)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococci log₂ MIC (mg/L)</td>
<td>2.8 ± 2.4</td>
<td>3.3 ± 2.5</td>
<td>3.3 ± 2.5</td>
<td>3.3 ± 2.5</td>
<td>0.78</td>
<td>-0.13</td>
<td>P = 0.6555</td>
</tr>
<tr>
<td>Enterococci log₂ MIC (mg/L)</td>
<td>1.8 ± 2.2</td>
<td>2.9 ± 2.1</td>
<td>3.3 ± 2.2</td>
<td>3.3 ± 2.2</td>
<td>1.9</td>
<td>-0.28</td>
<td>P = 0.0103</td>
</tr>
</tbody>
</table>

*Regression coefficients.
problems encountered in this pilot study, for example, selection of resistant
Gram-negative bacilli and Gram-positive cocci, the impact on colonisation resistance,
selection and last but not least, compliance.

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