Tissue and serum levofloxacin concentrations in diabetic foot infection patients


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Objectives: Levofloxacin has a high bioavailability and a broad antibacterial spectrum which covers the common pathogens found in acute and chronic diabetic foot infections. The purpose of our study was to determine the serum and tissue concentrations of levofloxacin when administered orally in patients with infected diabetic foot ulcers and to compare these values with microbiological findings.

Patients and methods: Ten outpatients with diabetes and ulcerations of the lower extremity were included. All patients received oral levofloxacin therapy at a dose of 500 mg once daily. Wound tissue and serum samples were collected and levofloxacin concentrations determined by HPLC with fluorescence detection. Additionally, microbiological cultures were performed from swabs and debrided wound tissue, both before and after treatment. MICs of levofloxacin for all bacterial isolates were determined using the Etest.

Results: Following oral treatment with levofloxacin for an average of 10 ± 3.8 days, all patients received debridement at the affected limbs. The levofloxacin concentrations in necrotic wound tissue were between 2.33–23.23 mg/kg and between 0.12–6.41 mg/L in serum. Tissue-to-serum ratios of levofloxacin concentrations for each patient were >1.0. The MIC values for all 17 initially isolated bacteria were ≤2 mg/L. In half of our patients, fluoroquinolones were one of the few oral monotherapy options where the spectrum covered all initially isolated pathogens.

Conclusion: Our data showed good tissue penetration of levofloxacin in diabetic foot ulcers. In combination with adequate surgical debridement, levofloxacin seems well suited to the treatment of skin structure infections of diabetics caused by susceptible organisms.

Keywords: quinolones, pharmacokinetics, soft tissue infections, diabetic foot infections, MRSA

Introduction

In antibiotic therapy, it is important to achieve effective local tissue concentration at the infected site. In diabetic foot infection (a lesion with reduced blood flow), this is particularly difficult to achieve and the diabetic foot syndrome represents a special and complex problem among late diabetic complications. Currently 4%–7% of diabetic patients develop a diabetic foot syndrome at some time after the diagnosis of diabetes.1 For these patients, polymicrobial skin structure infections caused by reduced immune reactivity combined with functional disturbance of microcirculation are frequently seen following small injuries.2 The infected necrotic lesions constitute an increased risk for developing osteomyelitic or septic complications with consequent...
amputations.¹ Thus, when initiating treatment an antimicrobial agent should be chosen which achieves good penetration into the site of this difficult-to-treat infection.

Levofloxacin, a fluoroquinolone with high bioavailability and extended half-life, has a broad antibacterial spectrum including enterobacteria, non-fermenters and Gram-positive cocci.² Members of these species are common pathogens in acute and chronic diabetic foot infections and therefore levofloxacin may be a suitable agent in the therapy of diabetic outpatients. To date, there are no data on levofloxacin concentrations in infected necrotic tissue in diabetic patients. The purpose of our study was to determine serum and tissue concentrations of levofloxacin following the oral administration of 500 mg, for at least 6 days, to patients with infected diabetic foot ulcers, and to correlate these values with microbiological findings.

Materials and methods

Patients

The protocol was approved by the Ethics Committee of the University of Heidelberg, Germany. All patients were required to give written informed consent. Patients with open ulcers of the lower limbs resulting from underlying diabetes were considered suitable for the study. All patients had lesions of Wagner grade 2 (deep ulcer/wound penetrating to tendon, bone or joint with infection in the surrounding soft tissue)³ and were treated in an outpatient setting. Exclusion criteria were (i) administration of another fluoroquinolone within 7 days before surgery, (ii) known allergy to fluoroquinolones, (iii) end-stage renal disease, (iv) pregnancy or lactation, (v) co-administration of probenecid, cimetidine or ferrotherapy. During the initial examination of the patient, debridement was performed and a wound swab taken from the base of the infected ulcer. A control examination was carried out after at least 6 days. Blood and tissue samples (second debridement) were taken. The excised tissue was used for a microbiological follow-up culture as well as for levofloxacin analysis.

Drug administration

All patients received levofloxacin orally at a dose of 500 mg once daily following the initial examination. Levofloxacin was given for 10 ± 3.8 days before the second debridement. The patients received no other antimicrobial agents and no other known oral medications that could interact with levofloxacin (sucralfate, theophylline, fenbufen, antacids, magnesium).

Blood and tissue sampling

Blood samples were collected in serum tubes simultaneously with wound tissue debridement. Serum was separated by centrifugation (2772g, 10 min) and stored at −25°C until analysis. Tissue specimens were wiped gently with dry gauze and immediately stored at −25°C until analysis.

Analytical method

Levofloxacin was determined by high performance liquid chromatography (HPLC) with fluorimetric detection as described by Boettcher et al.⁴ ⁵ ⁶ Briefly, tissue and serum samples spiked with the internal standard ciprofloxacin were prepared by protein precipitation with perchloric and phosphoric acid and methanol; tissue samples were homogenized with an Ultra Turrax dispenser. The compounds were separated on a reversed-phase column with an acid mobile phase containing triethylamine (water, methanol, triethylamine and ortho-phosphoric acid, 75/250/4/2.5, by vol., pH 3). The HPLC assay was linear over the usable concentration range (0.1–40 mg/L). The limit of quantification was determined as 0.01 mg/L, the limit of detection was 0.001 mg/L. The intra- and interday coefficients of variation were <5%.

Microbiology

All wound swabs and tissues were transferred to a microbiology laboratory and cultured aerobically and anaerobically. Culture, isolation and identification of the bacteria were performed according to standardized techniques. Susceptibility testing was performed using the microdilution method (break points according to NCCLS). The exact MICs for levofloxacin were determined using the Etest (VIVA, Hürt, Germany).

Results

Levofloxacin concentrations

Initially, 11 patients were enrolled in the study (six men, five women). One patient was withdrawn because of non-compliance. Evaluable patients included five men and five women with a mean age of 62.8 years. Seven patients were classified as having type 2 diabetes and two as type 1 (one unclassified). Diabetes was controlled by insulin in seven patients. The patients had a mean Hba1c of 6.82% (median 6.9%, range, 4.3%–7.6%) and an average serum creatinine of 1.28 mg/dL (113 μmol/L) [median 1.15 mg/dL (102 μmol/L), range, 0.7 (62) to 1.73 (153) mg/dL (μmol/L)]. Patients receiving 500 mg levofloxacin orally once a day had serum concentrations of 0.12–6.41 mg/L (Table 1). The levofloxacin concentrations in wound tissue were between 2.33–23.23 mg/kg. Concentrations in tissue were higher compared with serum in all patients (Table 1). There was no correlation or trend between tissue concentration of levofloxacin

<table>
<thead>
<tr>
<th>Patient</th>
<th>Levofloxacin concentration (mg/L or mg/kg)</th>
<th>Time from last dose to sampling (h)</th>
<th>Levofloxacin therapy (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3</td>
<td>6.41 7.22</td>
<td>2–4</td>
<td>8</td>
</tr>
<tr>
<td>D6</td>
<td>1.12 2.33</td>
<td>2–4</td>
<td>6</td>
</tr>
<tr>
<td>D7</td>
<td>2.42 15.76</td>
<td>2–4</td>
<td>14</td>
</tr>
<tr>
<td>D8</td>
<td>5.71 23.23</td>
<td>2–4</td>
<td>7</td>
</tr>
<tr>
<td>D9</td>
<td>1.09 15.36</td>
<td>2–4</td>
<td>13</td>
</tr>
<tr>
<td>D10</td>
<td>0.12 2.36</td>
<td>2–4</td>
<td>11</td>
</tr>
<tr>
<td>D11</td>
<td>5.42 9.66</td>
<td>2–4</td>
<td>14</td>
</tr>
<tr>
<td>D1</td>
<td>2.82 7.73</td>
<td>6–7</td>
<td>6</td>
</tr>
<tr>
<td>D2</td>
<td>NA 14.14</td>
<td>15–16</td>
<td>6</td>
</tr>
<tr>
<td>D5</td>
<td>0.67 10.02</td>
<td>23–24</td>
<td>15</td>
</tr>
<tr>
<td>Mean</td>
<td>2.86 10.78</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>s.D.</td>
<td>2.40 6.48</td>
<td></td>
<td>3.77</td>
</tr>
<tr>
<td>Median</td>
<td>2.42 9.84</td>
<td></td>
<td>9.5</td>
</tr>
</tbody>
</table>

NA, not available.
and time from last dose to sampling [correlation coefficient (Pearson): $r^2 = 0.06$, $P = 0.51$].

**Microbiology**

A microbiological swab was obtained from nine out of 10 patients before levofloxacin therapy. At least one potential pathogen was isolated from each of these nine patients (Table 2). Coagulase-negative staphylococci and corynebacteria were regarded as skin flora and non-pathogens. After levofloxacin therapy of 6–15 days the causal pathogen persisted in two patients (case D1 and D2). In one patient (case D11), a new pathogen (levofloxacin resistant) was isolated and in seven patients (cases D3–D10) no pathogen was found following treatment. In terms of the pharmacokinetic–pharmacodynamic correlation, the ratio between levofloxacin concentrations in wound tissue and the MIC of the causal pathogen was calculated. This ratio was in the range >1.2–82.2 for the pathogens isolated before levofloxacin therapy. In total, six of the nine patients with positive findings at the initial examination achieved microbiological cure.

**Discussion**

Levofloxacin showed excellent penetration into the wound tissue of the diabetic foot ulcers treated in this study, achieving concentrations, well in excess of those found in serum. These tissue concentrations were similar to those reported for ofloxacin in necrotic foot lesions of diabetic and non-diabetic patients with peripheral arterial occlusive disease.\(^7,8\) Chow \(et\ al.\)\(^9\) examined skin tissue of healthy volunteers with a dose of 750 mg levofloxacin once daily for 3 days. He found the highest concentration of levofloxacin 6h after administration of the dose, with a mean concentration of $11.87 \pm 2.58$ mg/kg. Our results for wound tissue in diabetic patients treated with 500 mg of levofloxacin, at $10.78 \pm 6.48$ mg/kg, were similar to the figures reported by Chow \(et\ al.\)\(^9\). However, unlike Chow and colleagues, at steady state, after at least 6 days of oral therapy with levofloxacin, we did not find a correlation between tissue concentration of levofloxacin and time from last dose to sampling (Table 1).

In this study, a variety of pathogens were isolated, including multi-resistant and non-fermenting bacteria. Microbiological follow-up cultures showed that in seven out of nine patients (78%) the pathogens initially isolated could be eradicated, although in a further patient, infection with a different organism occurred (Table 2). Our results are similar to those reported by Lipsky \(et\ al.\)\(^10\) who found that 78% of diabetic foot infections responded satisfactorily to a parenteral-to oral regimen of ofloxacin. A slightly higher microbiological eradication rate (83.4%) was reported by Graham \(et\ al.\)\(^11\) using 750 mg levofloxacin once daily for treatment of complicated skin structure infections in a larger study with 98 patients evaluable for microbiological efficacy.

In two cases, we found no change in isolated bacteria (in both cases the bacteria isolated before and after treatment were susceptible to levofloxacin). In one case, a methicillin-susceptible but levofloxacin-resistant *Staphylococcus aureus* was cultured following treatment. The tissue concentrations in these three patients were high (7.73, 9.66 and 14.14 mg/kg) and there did not appear to be a relationship between concentration and outcome. In 50% of our patients (D2, D6, D8, D10 and D11), fluoroquinolones were one of the few oral monotherapy options where the spectrum covered all of the isolated pathogens. Levofloxacin exhibits an adequate spectrum for the empirical treatment of diabetic foot ulcer. However, during focused therapy in the other 50% of our patients there would have been the possibility to use narrower-spectrum and less expensive agents.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Before levofloxacin therapy</th>
<th>MIC of levofloxacin (mg/L)</th>
<th>After levofloxacin therapy</th>
<th>MIC of levofloxacin (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td><em>S. aureus</em> (MSSA)</td>
<td>0.094</td>
<td><em>S. aureus</em> (MSSA)</td>
<td>0.094</td>
</tr>
<tr>
<td></td>
<td><em>Streptococcus agalactiae</em> (group B)</td>
<td>0.25</td>
<td><em>S. agalactiae</em> (group B)</td>
<td>0.25</td>
</tr>
<tr>
<td>D2</td>
<td><em>S. aureus</em> (MRSA)</td>
<td>2.0</td>
<td><em>S. aureus</em> (MRSA)</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>enterococci</td>
<td>0.25</td>
<td>enterococci</td>
<td>0.25</td>
</tr>
<tr>
<td>D3</td>
<td><em>S. aureus</em> (MSSA)</td>
<td>0.125</td>
<td>skin flora</td>
<td></td>
</tr>
<tr>
<td>D4</td>
<td><em>Citrobacter diversus</em></td>
<td>&lt;2.0</td>
<td>skin flora</td>
<td></td>
</tr>
<tr>
<td>D6</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>&lt;2.0</td>
<td>no growth</td>
<td></td>
</tr>
<tr>
<td>D7</td>
<td><em>S. aureus</em> (MSSA)</td>
<td>&lt;2.0</td>
<td>no growth</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Klebsiella species</em></td>
<td>&lt;2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D8</td>
<td><em>Proteus mirabilis</em></td>
<td>&lt;2.0</td>
<td>skin flora</td>
<td></td>
</tr>
<tr>
<td></td>
<td>enterococci</td>
<td>&lt;2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Stenotrophomonas maltophilia</em></td>
<td>&lt;2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>S. aureus</em> (MSSA)</td>
<td>&lt;2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D9</td>
<td>NA</td>
<td>NA</td>
<td>skin flora</td>
<td></td>
</tr>
<tr>
<td>D10</td>
<td><em>Bacteroides fragilis</em></td>
<td>&lt;2.0</td>
<td>skin flora</td>
<td></td>
</tr>
<tr>
<td></td>
<td>enterococci</td>
<td>&lt;2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D11</td>
<td><em>Acinetobacter baumannii</em></td>
<td>0.25</td>
<td><em>S. aureus</em> (MSSA)</td>
<td>&gt;32</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Pseudomonas spp</em></td>
</tr>
</tbody>
</table>

NA, not available; MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*.
In a recent publication, therapy with fluoroquinolones was found to be a significant risk factor in the acquisition of methicillin-resistant *S. aureus* (MRSA) by hospitalized patients. The authors of this study suggested that the eradication of susceptible microorganisms normally colonizing the skin and mucous membranes effectively opens an ecological niche and in doing so, renders a patient vulnerable to colonization by resistant strains endemic in the hospital, including MRSA. For this mechanism to succeed, MRSA needs to be present in the environment ready to colonize the patient given the opportunity. If this is correct, it is unlikely that the mechanism also operates in a setting with lower MRSA prevalence in patients and lower MRSA contamination in the environment. At present, there are no data to suggest that where the prevalence of MRSA is low, fluoroquinolone use for therapy of diabetic foot infection constitutes an increased risk for MRSA acquisition. In conclusion, we have shown that levofloxacin reaches high concentrations in infected and necrotic wound tissue of diabetic foot ulcers. In combination with adequate surgical debridement, levofloxacin seems well suited to the treatment of skin structure infections of diabetics, caused by susceptible organisms.

**Acknowledgements**

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**References**