

Comparative evaluation of oral levofloxacin and parenteral nafcillin in the treatment of experimental methicillin-susceptible *Staphylococcus aureus* osteomyelitis in rabbits

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Methicillin-susceptible *Staphylococcus aureus* (MSSA) is the most common pathogen recovered from osteomyelitis patients. The current standard therapeutic method for acute phase osteomyelitis is parenteral antibiotic therapy. However, parenteral administration has negative aspects, such as secondary infection, patient inconvenience and high cost. The use of single oral antibiotic therapy may alleviate these problems. Therefore, the purpose of this study was to compare the effectiveness of standard once per day dosing of oral levofloxacin with a standard parenteral antibiotic regimen (nafcillin four times daily) for the treatment of experimental MSSA osteomyelitis in rabbits. Nearly all tibiae from untreated infected controls ($n = 27$) revealed positive cultures (93%) for *S. aureus*, while the levofloxacin-treated group ($n = 20$) demonstrated significantly lower percentages of *S. aureus* infection (50%). The infected tibiae of the nafcillin-treated group ($n = 20$) demonstrated significantly lower percentages (10%) of infected tibiae than either the controls or the levofloxacin-treated groups ($P < 0.05$). The inferior efficacy of levofloxacin may have been due to the pharmacokinetic profile of this fluoroquinolone. The serum kinetics demonstrated that following single dose administration, levofloxacin was almost undetectable after 12 h. Studies in which levofloxacin is dosed every 12 h or given at increased doses in order to obtain bactericidal concentrations throughout the treatment regimen are needed.

Introduction

Methicillin-susceptible *Staphylococcus aureus* (MSSA) causes a variety of infections, including osteomyelitis.^{1–4} Debridement and parenteral antibiotics lasting from 4 to 6 weeks remain the cornerstones of osteomyelitis therapy.⁵ Because of the ease of administration, oral antibiotic treatment would represent a significant advantage compared with a lengthy parenteral regimen as the many problems associated with parenteral antibiotic administration, such as secondary line and site infections, patient inconvenience and high cost, may be averted.⁶ However, the use of single-agent oral therapy in experimental MSSA animal models has demonstrated only moderate effectiveness in sterilizing bone. This lack of effectiveness has been a result of the

inability of most oral antibiotics to achieve concentrations in the bone significantly above their respective MICs.

The quinolone class of antibacterial agents has been used in the treatment of MSSA and methicillin-susceptible *Staphylococcus epidermidis* (MSSE) osteomyelitis.^{7,8} Levofloxacin is a third-generation quinolone (refer to Wimer *et al.*⁹ for a therapeutic review of levofloxacin). The antibiotic has been derived from the D and L racemic mixture ofloxacin in which the D-ofloxacin stereoisomer (which provides no antimicrobial activity and much of the ofloxacin-associated toxicity) has been removed. It has an exceptionally broad spectrum of antibacterial activity against Gram-positive, Gram-negative and anaerobic bacteria.¹⁰ While levofloxacin has only 25% of the killing efficacy against *Pseudomonas aeruginosa*, increased activity against *S. aureus*,

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S. epidermidis, *Streptococcus* spp. and various anaerobes, the low incidence of development of microbial resistance, the high serum and bone concentrations and lack of significant toxicity, provide a distinct advantage over the existing second- and third-generation quinolones.⁹ Levofloxacin has a long serum half-life and is currently given once a day in clinical practice.¹⁰ Therefore, levofloxacin may be an ideal agent for the treatment of osteomyelitis.

In a recent clinical study, oral levofloxacin, lomefloxacin and ciprofloxacin were evaluated for treatment of chronic osteomyelitis.¹¹ Specifically, in the levofloxacin-treated group, infection was undetected and no relapses in infection occurred in nine of 15 patients (60%). The authors concluded that oral fluoroquinolones could be used as safe and effective antimicrobial therapy if given for a prolonged course and coupled with adequate surgical debridement. One weakness with this study is that it depended upon a lengthy antibiotic course of therapy (average of 60.6 days). Also, cure rates with oral fluoroquinolones were not compared with the gold standard of parenteral therapy that usually results in infection clearance rates of 80–90%.¹² Another recent clinical study used ofloxacin for the treatment of chronic osteomyelitis and reported a 91% cure rate.¹³ Besides the choice of antibiotic, this study differed in the utilization of a twice daily dosing regimen. Therefore, the purpose of this study was to compare the effectiveness of standard once per day dosing of oral levofloxacin with a standard parenteral antibiotic regimen (nafcillin dosed four times daily) for the treatment of experimental MSSA osteomyelitis.^{14–18}

Materials and methods

Organism

The strain of *S. aureus* was obtained from a patient with osteomyelitis undergoing treatment at The University of Texas Medical Branch, Galveston, TX, USA. The strain was an MSSA phage type 52/52A/80 and was stored at –70°C in defibrinated sheep blood. Antibiotic tube dilution sensitivities¹⁹ were measured for levofloxacin and nafcillin in a cation-supplemented Mueller–Hinton broth (CSMHB).

Production of osteomyelitis

The bacterium was grown overnight in tryptic soy broth and diluted in saline to a concentration of 1.0×10^7 cfu/mL. New Zealand White female rabbits, 8 weeks of age and weighing 1.5–3.0 kg, were used. All procedures were performed as per humane criteria set forth by University of Texas Medical Branch Animal Care and Use Committee. Rabbits were anaesthetized using an intramuscular injection of 30 mg/kg ketamine (Ketaset; Fort Dodge Laboratories, Inc., Fort Dodge, IA, USA), 10 mg/kg acepromazine

(Fort Dodge Laboratories), and 1 mg/kg xylazine (Rugby Laboratories, Inc., Rockville Center, NY, USA). An 18-gauge needle was inserted percutaneously through the lateral aspect of the left tibial metaphysis into the intramedullary cavity. Sodium morrhuate (Eli Lilly, Indianapolis, IN, USA) [0.1 mL of a 5% (w/v) solution], 0.1 mL of *S. aureus* (1.0×10^6 cfu) and 0.2 mL of sterile saline were injected sequentially.^{14–18}

Therapeutic trials

The rabbits ($n = 70$) were randomized into three groups at the time of infection (day zero), and treatment began 14 days later. Group 1 rabbits ($n = 30$) were infected but untreated and were included as controls. Three of these rabbits died before the end of the study, leaving a total of 27 rabbits that could be evaluated. Group 2 ($n = 20$) received oral levofloxacin (c. 30 mg/kg) every 24 h dissolved in 0.5% methyl cellulose,²⁰ group 3 ($n = 20$) received parenteral subcutaneous nafcillin 30 mg/kg every 6 h.¹⁵ The antibacterial agents were given from day 14–42 (28 days total). Oral levofloxacin was administered to the animals orally via a syringe coated with sugar,²⁰ and nafcillin was administered by subcutaneous injection into the back of the rabbits' necks.¹⁴ Following completion of treatment, the rabbits were observed for 2 weeks before killing, in order to allow for the re-growth of any remaining *S. aureus*. The rationale behind using subcutaneous versus intravenous dosing was two-fold. First, intravenous dosing in the rabbit is very problematic owing to the lack of available vessels and the inability to maintain an intravenous catheter for any length of time in the unanaesthetized rabbit. In addition, subcutaneous injection is able to reach peak serum concentrations within minutes of injection at levels comparable to those seen in intravenous injections in the rabbit.

Roentgenograms of both tibiae were taken at antibiotic initiation (day 14) and at antibiotic termination (day 42). Severity of the infection by roentgenographic appearance was graded by a rating system reported previously (Table).¹⁵ The rabbits were weighed before infection and twice weekly until they died or were killed.

Bone cultures

Rabbits that died before treatment began at day 14 were not included in the study. At the conclusion of the study, rabbits were killed by an intravenous injection of sodium pentobarbital. Both tibiae were removed, dissected free of all soft tissue and processed for bacterial cultures. Using a 5.0 mm, single-action rongeur, the bones were split into small pieces and the marrow was removed. The whole bone was then pulverized and suspended in 3 mL of sterile 0.85% saline per gram of bone. Serial 10-fold dilutions were performed and streaked onto a tryptic soy agar blood plate for quantitative counts.

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Table. Criteria for grading the severity of *Staphylococcus aureus* osteomyelitis in rabbits

Gross pathology	Radiographic findings
0 normal	0 normal
1+ no bone involvement; soft-tissue swelling at proximal tibial metaphysis	1+ elevation or disruption of periosteum, or both; soft-tissue swelling
2+ soft-tissue abscess; <10% widening of proximal tibial metaphysis	2+ <10% disruption of normal bone architecture
3+ >10% widening of proximal tibial metaphysis	3+ 10–40% disruption of normal bone architecture
4+ disruption or pitting of normal bone architecture	4+ >40% disruption of normal bone architecture

Drug kinetics in serum and simultaneous level measurements in serum and bone

A group of uninfected animals and a group of 3–4 infected animals were given a single oral dose of levofloxacin (approximately 30 mg/kg) dissolved in 0.5% methyl cellulose solution,²⁰ or a single 30 mg/kg subcutaneous dose of nafcillin.¹⁴ Serum concentrations for levofloxacin and nafcillin were determined from blood drawn at 1, 2, 6, 12 and 24 h after dosing. In another group of uninfected and 3–4 infected animals, simultaneous serum and bone concentrations were determined 2 h after a single oral dose of levofloxacin or a single subcutaneous dose of nafcillin.

Drug assays in serum and bone

A disc diffusion bioassay using *Bacillus subtilis* (ATCC 6633) and *Sarcina lutea* (ATCC 9341) as the test organisms were used to measure levofloxacin and nafcillin concentrations, respectively, in both serum and bone eluates. The lower limits of detection for both antibiotics by these assays were determined. Standards of levofloxacin and nafcillin were stored at 1000 mg/L at –20°C and prepared with known concentrations of the antibiotic diluted in 100% normal rabbit serum. Twenty microlitre serum standards or serum samples were placed on blank discs (0.25 inch; Difco Laboratories, Detroit, MI, USA), plated on the seeded agar plates, and incubated at 37°C overnight. The diameters of the zones of inhibition of test bacteria growth were measured for both samples and standards. Unknown concentrations were determined from semi-log standard curves. Blood was drawn from each rabbit before the antibiotic administration, and if inhibitors were detected, the animal was not used in the study.

Infected or uninfected bones were prepared for assay by dissecting them free of all soft tissue, breaking them into small pieces, and removing the marrow. The bone was crushed, weighed and suspended in 50% 0.1 M sodium phosphate buffer (pH 7.5) and 50% normal rabbit serum. One millilitre of the buffer–serum solution was used per 0.5 g bone. The crushed bone was shaken (200 rpm) in a

50 mL conical centrifuge tube for 2 h at 4°C. Antibiotic in the supernatant fluid was assayed by the previously described disc diffusion methods. Standard solutions of the drugs were prepared by adding normal uninfected bone to the buffer–serum solution containing known amounts of either levofloxacin or nafcillin.

Fisher's exact test was used to compare the numbers of infected rabbits in each group. In order to determine whether there was a significant difference in bacterial concentrations in the bones of treated but still infected animals at the end of the study compared with untreated, infected controls, we used a two-tailed Student's *t*-test. This test was also used to compare the radiographic scores between the first (14 days post-infection) and second set of X-ray films (42 days post-infection). Differences between groups were deemed statistically significant if $P \leq 0.05$.

Results

MICs, MBC and radiographic evaluation

For the strain of *S. aureus* used in the experiment, the MIC and minimum bactericidal concentration (MBC) values for levofloxacin were 0.39 and 1.56 mg/L, respectively, while MIC and MBC values for nafcillin were <0.39 and 0.78 mg/L, respectively. No significant differences were observed between the first (14 days post-infection) and second set of X-ray films (42 days post-infection) for any of the control or experimental groups. This may be expected since radiographs often lag at least 2 weeks behind clinical progress.²¹

Serum pharmacokinetics and simultaneous bone and serum antibiotic levels

Concentrations of levofloxacin (30 mg/kg) and nafcillin (30 mg/kg) in the sera of infected and uninfected rabbits after administration of the respective drugs are shown in Figure 1 (a and b). Elimination of the antibiotics from the serum was fastest for nafcillin, followed by levofloxacin.

While nafcillin clearance was quite rapid, the four times per day dosage regimen enabled bactericidal concentrations to be maintained in the serum during the entire treatment phase of the study. However, levofloxacin was nearly undetectable by 12 h and was undetectable by 24 h. Since this antibiotic is only given once daily, the antibiotic concentrations fell below the MIC.

Figure 2 compares simultaneous concentrations in serum, non-infected bone and infected bone of animals 2 h after single-dose oral antibiotic administration of levofloxacin (30 mg/kg), and following a single subcutaneous dose of nafcillin (30 mg/kg). Comparable antibiotic concentrations were found in the bones of the nafcillin and the levofloxacin groups. However, the serum concentrations obtained in the nafcillin group were approximately 1.6 times those levels found in the levofloxacin group.

Bone cultures

Nearly all tibias from untreated infected controls ($n = 27$) revealed positive cultures (93%) for *S. aureus* (see Figure 3a). When compared with untreated controls, the levo-

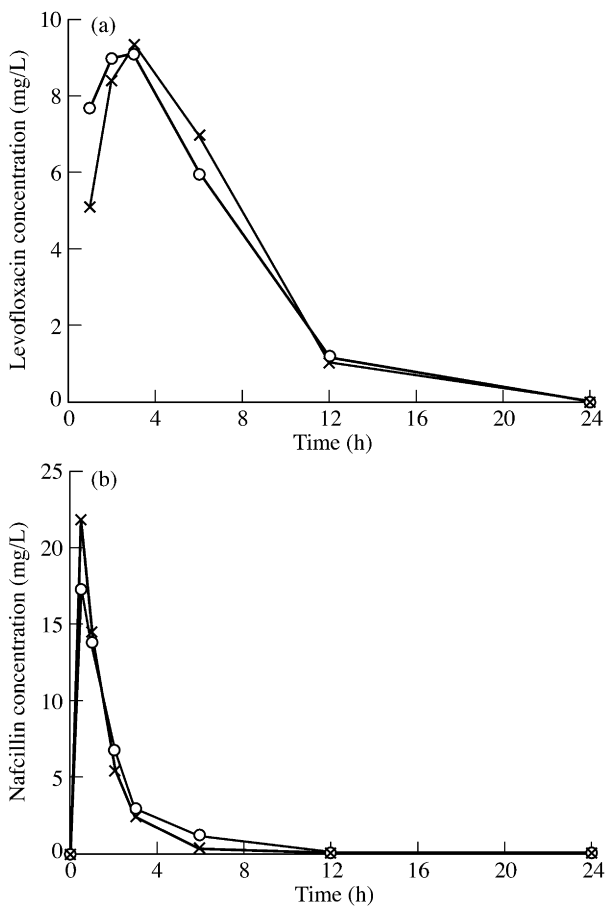


Figure 1. Serum concentrations of (a) levofloxacin (30 mg/kg dose) and (b) nafcillin (30 mg/kg) in infected (×; $n = 6$) and uninfected (○; $n = 6$) animals 0, 1, 3, 6, 12 and 24 h post-antibiotic administration.

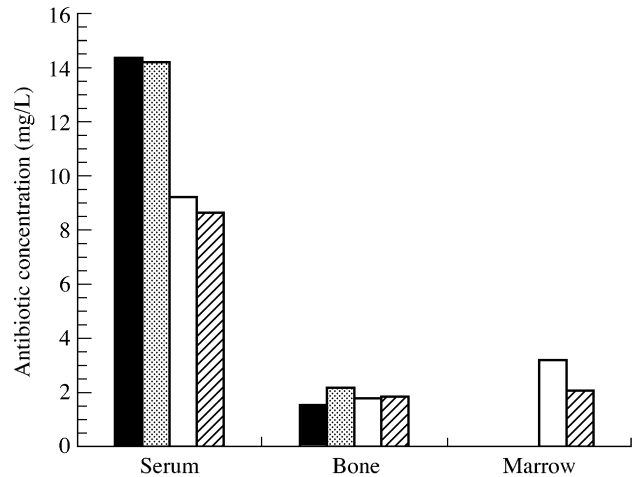


Figure 2. Simultaneous serum and bone concentration of levofloxacin (30 mg/kg) and nafcillin (30 mg/kg) in infected ($n = 6$) and uninfected rabbits ($n = 6$) 2 h after single-dose oral antibiotic administration. Nafcillin (non-infected), ■; nafcillin (infected), ▨; levofloxacin (non-infected), □; levofloxacin (infected), ▩. Marrow concentrations of nafcillin were not determined.

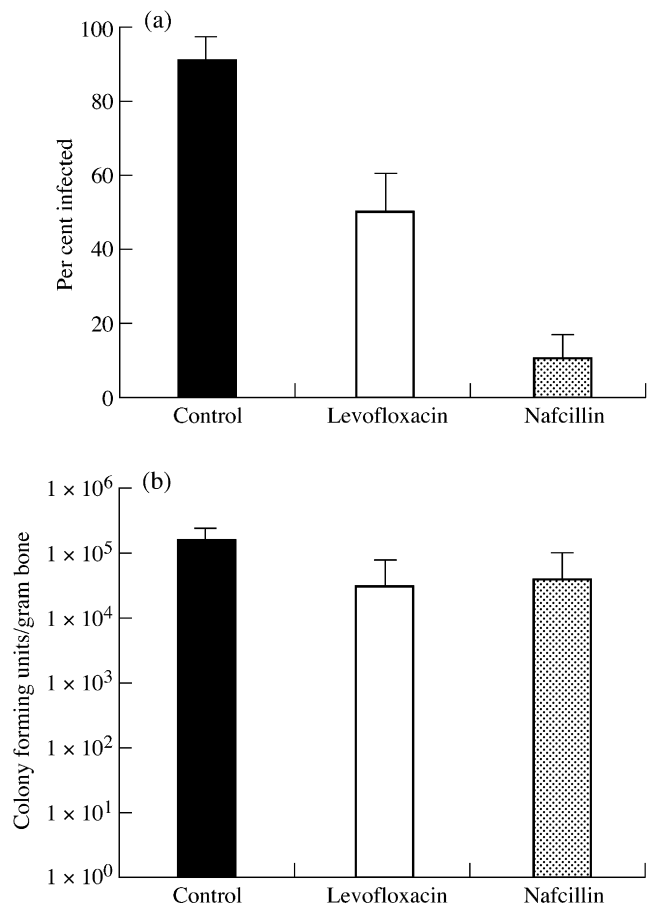


Figure 3. (a) Per cent of animals found with positive *S. aureus* cultures detected in tibias of control ($n = 27$), levofloxacin-treated rabbits ($n = 15$), and nafcillin ($n = 15$) groups and (b) the mean cfu levels in the bones (in cfu/g bone) of ofloxacin treated but still infected rabbits, nafcillin treated but still infected, and infected, untreated rabbits.

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floxacin group ($n = 20$) demonstrated significantly lower percentages of positive *S. aureus* infection (50%) ($P < 0.05$). The tibias of the nafcillin-treated group ($n = 20$) demonstrated significantly lower percentages (10%) of infected tibias than either the controls or the levofloxacin groups ($P < 0.05$). When the bacterial concentrations in the bones of levofloxacin-treated but still infected rabbits ($2.8 \times 10^4 \pm 5.7 \times 10^4$ cfu/g bone), nafcillin-treated but still infected ($3.6 \times 10^4 \pm 5.1 \times 10^4$ cfu/g bone) and infected, untreated rabbits ($1.5 \times 10^5 \pm 6.1 \times 10^4$ cfu/g bone) were compared, no statistically significant differences were observed ($P > 0.05$) (see Figure 3b).

Discussion

Levofloxacin, a third-generation fluoroquinolone, demonstrated a significant reduction in percentage infection in rabbits with tibial MSSA osteomyelitis when compared with non-treated, infected controls. However, this quinolone demonstrated inferior efficacy in reducing the *S. aureus* concentrations to undetectable levels when compared with nafcillin, a standard parenteral antibiotic used in the clinical treatment of MSSA osteomyelitis. When the bacterial concentrations in the bones of levofloxacin-treated but still infected rabbits, nafcillin-treated but still infected, and infected, untreated rabbits were compared, no statistically significant differences were observed.

The inferior efficacy of levofloxacin in the resolution of MSSA osteomyelitis seen in this study may have been caused by three factors. First, the *in vivo* efficacy of the nafcillin and levofloxacin may have differed owing to the different mechanisms of action of the two antibiotics, namely cell wall formation inhibition versus DNA gyrase and topoisomerase IV inhibition, respectively. Secondly, the levofloxacin MIC (0.39 mg/L) and MBC (1.56 mg/L) values for the strain of MSSA used in this study differed by a factor of four. Since an MIC/MBC ratio that approximates 1.0 is most likely to be conducive to the resolution of osteomyelitic infections, this difference may have detracted from the efficacy of this fluoroquinolone. Lastly, the serum kinetics demonstrated that following single dose administration, the levofloxacin was nearly undetectable after 12 h. Therefore, the once per day dosing schedule of the levofloxacin rabbit group may have not been sufficient to maintain bactericidal concentrations within the infected tibias of rabbits.

In order to determine accurately the efficacy of levofloxacin in the treatment of osteomyelitis, additional studies may be required. Other studies might investigate a dosing schedule of levofloxacin every 12 h instead of the once daily dosing schedule used in this study, as was suggested in a recent clinical study for severe community-acquired pneumonia.²² Another option is to increase the dosage of levofloxacin in order to obtain bactericidal concentrations throughout the treatment regimen. In addition,

studies that involve using levofloxacin in combination with other antibiotics in order to obtain synergic activity and prevention of rapid antibiotic resistance development may be warranted. *In vitro* synergic activity against *S. aureus* can be obtained when levofloxacin is coupled with oxacillin, ceftazadime, tobramycin and ampicillin.^{23–25} However, other recent *in vitro* studies demonstrated an indifference or antagonism using a combination of levofloxacin and rifampicin or β -lactams in treating serious pneumococcal infections.²⁶ Therefore, caution must be exercised and further studies must be performed to elucidate accurately the efficacy of this third-generation fluoroquinolone.

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