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Linezolid Therapy of *Staphylococcus aureus* Experimental Osteomyelitis

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The in vivo activity of linezolid or cefazolin against a clinical isolate of methicillin-susceptible *Staphylococcus aureus* (linezolid MIC, 2 μg/ml) was studied in a rat model of experimental osteomyelitis. Sixty rats with experimental *S. aureus* osteomyelitis were treated for 21 days with no antimicrobial, with 25 μg of linezolid per kg of body weight administered intraperitoneally twice or three times a day, or with 50 μg of cefazolin per kg administered intramuscularly three times a day. After treatment, the animals were sacrificed and the infected tibiae were processed for quantitative bacterial cultures. The results of treatment were expressed as $\log_{10}$ CFU/gram of bone and analyzed by rank sum analysis. The results of linezolid treatment were not significantly different from those of untreated controls, while cefazolin treatment was significantly more active than no treatment or linezolid treatment.

Staphylococcus aureus is a common cause of chronic osteomyelitis. Treatment of chronic *S. aureus* osteomyelitis in adults typically consists of surgical debridement and prolonged therapy with an intravenous antimicrobial agent active against the infecting *S. aureus* strain. Administration of prolonged courses of intravenous antimicrobial therapy is costly and inconvenient and requires long-term intravenous access, which may be fraught with complications (e.g., line sepsis). The use of a highly active oral antistaphylococcal agent for the treatment of chronic *S. aureus* osteomyelitis is attractive. The quinolones, in combination with rifampin, have been successfully used for the treatment of orthopedic implant-related staphylococcal infections (8), but staphylococcal resistance to quinolones may limit the role of these agents for bone and joint infections.

Oxazolidinones are a new class of synthetic antimicrobial agents which inhibit the initiation of protein synthesis. Linezolid is an orally and intravenously administered oxazolidinone which is active in vitro against *S. aureus* (7). Recently, successful treatment with intravenous followed by oral linezolid of two humans with methicillin-resistant *S. aureus* intraabdominal infection and parotitis has been reported (1, 2). There are no published data on the activity of linezolid in bone and joint infections. If effective against chronic *S. aureus* osteomyelitis, oral linezolid may be a useful agent for this indication. The purpose of this study was to examine the activity of linezolid in a rat model of chronic methicillin-susceptible *S. aureus* osteomyelitis.

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Twenty-three clinical methicillin-susceptible *S. aureus* isolates were studied in vitro. The MICs and minimal bactericidal concentrations (MBCs) of linezolid and cefazolin were determined using a microtube dilution technique according to National Committee for Clinical Laboratory Standards guidelines with an inoculum of 5×10^5 CFU/ml (5). The MICs at which 90% of the isolates tested were inhibited (range) were 8 μg/ml (2 to 16 μg/ml) for linezolid and 1 μg/ml (0.5 to 2 μg/ml) for cefazolin. The MBCs at which 90% of strains tested were killed (range) were >128 μg/ml (16 to > 128 μg/ml) for linezolid and 8 μg/ml (1 to 64 μg/ml) for cefazolin. One strain was chosen for in vivo studies. The MICs were 2 μg/ml for linezolid and 1 μg/ml for cefazolin for the strain used for in vivo study. The MBCs were >128 μg/ml for linezolid and 1 μg/ml for cefazolin for the strain used for in vivo study. Linezolid exhibited bacteriostatic activity against the study strain.

Experimental osteomyelitis was established in male Wistar rats using a modification of Zak's model of experimental osteomyelitis (6). Animals were anesthetized with ketamine and xylazine, and the proximal third of the left tibia was surgically exposed. A 0.5-mm hole was drilled into the medullary cavity. Fifty microliters of morrhuate sodium (a sclerosing agent) followed by 50 μl of a bacterial inoculum containing 10^8 CFU of *S. aureus* per ml was injected into the bone. The hole was sealed with dry dental gypsum, the skin was closed with surgical staples, and the wound was sprayed with antiseptic plastifilm.

Four weeks after initiation of infection the counts of *S. aureus* per gram of bone were 5.68, 5.76, and 6.24 $\log_{10}$ CFU in the three animals in which this was studied. Four weeks after initiation of infection, systemic antimicrobial therapy was initiated in the remainder of the animals and was continued for 21 days. The numbers of animals in each treatment group are outlined in Table 1. Twelve hours after completion of antimicrobial therapy, the rats were sacrificed with a lethal dose of pentobarbital. The infected left tibiae were aseptically removed, weighed, and frozen. The frozen tibiae were pulverized to a fine powder; the powder was suspended in 2 ml of tryptic soy broth and serially diluted in tryptic soy broth. Aliquots (0.1

TABLE 1. Outcome of therapy of chronic osteomyelitis due to *S. aureus*[a]

Antimicrobial therapy	No. of animals	Median $\log_{10}$ CFU/g of bone	Range (25th–75th percentile)
None	16	5.09	4.59–5.49
Linezolid b.i.d.	16	5.32	4.54–5.75
Linezolid t.i.d.	13	4.90	3.03–5.70
Cefazolin	15	3.78	2.81–4.36

[a] Linezolid dosed b.i.d, was not significantly different than no treatment; linezolid dosed t.i.d. was not significantly different than no treatment or cefazolin. Cefazolin was more active ($P = 0.001$) than no treatment or linezolid dosed b.i.d.

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TABLE 2. Concentrations of antimicrobials in serum during experimental infection

Drug, dose, and route	Concn in serum (μg/ml) at:						AUC_{0-8} (μg · h/ml)
	0.5 h	1 h	2 h	4 h	6 h	8 h	
Linezolid, 25 mg/kg, i.p.[a]	32.0 ± 1.5	18.5 ± 4.1	8.7 ± 2.5	5.8 ± 0.5	4.4 ± 0.6	<1 ± 0.01	—[d]
Linezolid, 25 mg/kg, i.p.[b]	16.5 ± 6.2	21.4 ± 4.2	11.7 ± 1.0	7.6 ± 0.6	3.7 ± 3.0	1.1 ± 0.6	61.5
Cefazolin, 50 mg/kg, i.m.[c]	163 ± 15.5	82.5 ± 9.5	14.9 ± 5.7	<5 ± 0.01	<5 ± 0.01	<5 ± 0.01	110

[a] Mean concentration in serum among three healthy rats after a single dose of linezolid.
[b] Mean concentration in serum among three infected rats at steady state after 6 days of linezolid administered t.i.d.
[c] Mean concentration in serum among three infected rats at steady state after 5 days of cefazolin administered t.i.d. intramuscularly (i.m.).
[d] For a single dose of linezolid, 25 mg/kg, i.p., the AUC_{0-6} was 52.5 μg · h/ml.

ml) of each dilution were plated onto the surfaces of 5% sheep blood agar plates and incubated for 48 h at 35°C in 5% CO_2. The plates were examined for purity and colony morphology. The colonies were counted and the $\log_{10}$ CFU/gram of tibia was algebraically calculated. The results of treatment were expressed as the median $\log_{10}$ CFU/gram of bone for each treatment group.

Linezolid powder was dissolved in sterile water and administered intraperitoneally (i.p.) at doses of 25 mg/kg of body weight twice daily (b.i.d.) or three times daily (t.i.d.). Cefazolin (Marsam Pharmaceuticals Inc., Cherry Hill, N.J.) was administered intramuscularly at a dose of 50 mg/kg t.i.d. Untreated control rats were included in each experiment.

The concentration in serum of the antimicrobials used in vivo was determined for three rats 30 min and 1, 2, 4, 6, and 8 h after administration of a single dose of linezolid and on the fifth day of treatment (for cefazolin) or the sixth day of treatment (for linezolid; 25 mg/kg t.i.d.). Blood was obtained via heart puncture. Antimicrobial concentrations were determined by high-performance liquid chromatography (for linezolid) or bioassay (for cefazolin) (4). Bioassays were performed in triplicate on Mueller-Hinton agar seeded with *Bacillus subtilis* ATCC 6633 as the indicator organism. Paper disks with 20 μl of serum were placed on the bioassay plates and incubated for 16 to 18 h in room air at 30°C. The zone sizes were measured with calipers, and concentrations were calculated against a five-point standard curve by linear regression. Concentrations in serum of linezolid and cefazolin with the different treatment regimens are shown in Table 2. Peak serum linezolid concentrations were similar to those documented for humans. Serum linezolid concentrations were above the MIC for 75% of the 8-h dosing interval, and the area under the concentration-time curve from 0 to 24 h (AUC_{0-24}) was above 100 μg · h/ml.

Results of treatment of *S. aureus* experimental osteomyelitis are shown in Table 1. One rat from the cefazolin treatment group and three rats from the group dosed with linezolid b.i.d. were lost to cardiac puncture and were excluded from the treatment data analysis. No animals had sterile bones. Differences in median $\log_{10}$ CFU of *S. aureus* per gram of bone among the different treatment groups were analyzed using the Wilcoxon rank sum test. Linezolid dosed b.i.d. was not significantly different than no treatment; linezolid treatment dosed t.i.d. was not significantly different than no treatment or cefazolin treatment. Cefazolin treatment was more active ($P = 0.001$) than no treatment or linezolid treatment dosed b.i.d. in this model.

Samples of *S. aureus* recovered from quantitative tissue cultures were tested for emergence of resistance to the antimicrobial agent used to treat that animal as follows. Each colony of *S. aureus* derived from the broth dilution of pulverized tibia which resulted in 10 to 100 colonies of *S. aureus* on the associated agarose plate was touched with the end of a sterile wooden stick. The stick was then used to inoculate a microtube dilution assay using the associated antimicrobial agent (5). The MICs after treatment were within one dilution of the pretreatment MICs; therefore, no emergence of resistance was detected in organisms recovered from any animal.

Linezolid was previously shown to be modestly active in a murine methicillin-susceptible *S. aureus* soft tissue infection model (3). The amount of oral linezolid required to eradicate *S. aureus* from 50% of abscesses was 39.0 mg/kg per dose administered b.i.d. (versus 4.7 mg/kg per dose of subcutaneous vancomycin administered b.i.d.). In a murine model of lethal infection in which methicillin-susceptible *S. aureus* was injected i.p., the 50% effective dosage of linezolid administered orally at 1 and 5 h postinfection was 2.9 to 3.7 mg/kg/day (3).

We studied the activity of linezolid in a rat model of chronic *S. aureus* osteomyelitis, because analogous infections in humans are common in clinical practice and typically require prolonged courses of intravenous antimicrobial therapy (and aggressive surgical debridement) for successful treatment. The excellent oral bioavailability of linezolid and its activity in vitro against *S. aureus* make this agent a potentially attractive therapeutic option for chronic *S. aureus* osteomyelitis in humans. In our study, linezolid treatment was no different than no treatment in a rat model of chronic *S. aureus* osteomyelitis. The rat model of chronic *S. aureus* osteomyelitis is a rigorous test of agents for antimicrobial activity against *S. aureus*. Whether or not the activity of linezolid in our rat model of chronic osteomyelitis would be improved by the addition of rifampin is unknown. Cefazolin was active against methicillin-susceptible *S. aureus* in this model and is representative of the activity of an antibiotic with known moderate efficacy for the treatment of methicillin-susceptible *S. aureus* bone and joint infections.

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REFERENCES

1. **Antony, S. J., K. M. Bitter, T. Moreland, F. Raudales, and H. Diaz-Luna.** 1999. Methicillin-resistant *Staphylococcus aureus* infection in a renal allograft recipient treated successfully with a novel new antimicrobial agent (Linezolid): new treatment options for infections due to resistant organisms. Clin. Infect. Dis. **29:**1341–1342.
2. **Chien, J., M. Kucia, and R. Salata.** 2000. Use of linezolid, an oxazolidinone, in the treatment of multidrug-resistant Gram-positive bacterial infections. Clin. Infect. Dis. **30:**146–151.
3. **Ford, C. W., J. C. Hamel, D. M. Wilson, J. K. Moerman, D. Stapert, R. J. Yancey, Jr., D. K. Hutchinson, M. R. Barbachyn, and S. J. Brickner.** 1996. In vivo activities of U-100592 and U-100766, novel oxazolidinone antimicrobial agents, against experimental bacterial infections. Antimicrob. Agents Chemother. **40:**1508–1513.
4. **Klassen, M., and S. Edberg.** 1996. Measurement of antibiotics in human body fluids: techniques and significance, p. 230–240. *In* V. Lorian (ed.), Antibiotics in laboratory medicine, 4th ed. Williams & Wilkins, Baltimore, Md.
5. **National Committee for Clinical Laboratory Standards.** 1997. Performance standards for antimicrobial susceptibility testing. M7-A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.

6. **O'Reilly, T., and J. T. Mader.** 1999. Rat model of bacterial osteomyelitis of the tibia, p. 560–575. *In* O. Zak and M. A. Sande (ed.), Handbook of animal models of infection, experimental model in antimicrobial chemotherapy. Academic Press, San Diego, Calif.
7. **Patel, R., M. S. Rouse, K. E. Piper, and J. M. Steckelberg.** 1999. In vitro activity of linezolid against vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus* and penicillin-resistant *Streptococcus pneumoniae*. Diagn. Microbiol. Infect. Dis. **34:**119–122.
8. **Zimmerli, W., A. F. Widmer, M. Blatter, R. Frei, and P. E. Ochsner.** 1998. Role of rifampin for treatment of orthopedic implant-related staphylococcal infections: a randomized controlled trial. Foreign-Body Infection (FBI) Study Group. JAMA **279:**1537–1541.