

Oral Rifampin Plus Azithromycin or Clarithromycin to Treat Osteomyelitis in Rabbits

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A rabbit model for *Staphylococcus aureus* osteomyelitis was used to compare 28-day combination antibiotic therapy using oral rifampin (40 mg/kg, twice daily) plus oral azithromycin (50 mg/kg, once per day), oral clarithromycin (80 mg/kg, twice daily), or parenteral nafcillin (30 mg/kg, four times daily). The left tibial metaphysis of New Zealand White rabbits was infected with *Staphylococcus aureus*. Grades 3 to 4 osteomyelitis (according to the Cierny-Mader classification system) development in the rabbits was confirmed radiographically. After antibiotic therapy regimens of 28 days, all tibias from controls that were infected but left untreated (n = 10) revealed positive cultures for *Staphylococcus aureus* at a mean concentration of 2.8×10^4 colony forming units/g bone. The rifampin plus clarithromycin (n = 15) and rifampin plus azithromycin (n = 15)

groups showed significantly lower percentages of positive *Staphylococcus aureus* infection (20% and 13.3%, respectively) and bacterial concentrations (3.5×10^1 and 1.75×10^1 colony forming units/g bone, respectively). The osteomyelitic tibias of the nafcillin plus rifampin treated group (n = 7) showed no detectable *Staphylococcus aureus* infection (significantly lower than controls). The differences observed for bone bacterial concentrations and sterilization percentages between the antibiotic treated groups were not statistically significant. Although fluoroquinolones (including ofloxacin and ciprofloxacin) are the agents usually prescribed with rifampin, increasing resistance has been observed. Although macrolides traditionally are not used in the treatment of osteomyelitis, the results of this study indicate that azithromycin and clarithromycin may be attractive partners for rifampin for the treatment of *Staphylococcus aureus* osteomyelitis in humans.

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Methicillin sensitive *Staphylococcus aureus* is the most common organism isolated from patients with osteomyelitis.^{1,28} Patients with osteomyelitis are treated with debridement surgery, dead space management, and antibiotics. Oral antibiotic therapy is being used more commonly for the treatment of osteomyelitis. Patients usually are given 2 weeks of parenteral therapy, followed by 4 to

6 weeks of oral antibiotic therapy. Because of the ease of administration, oral antibiotic treatment represents a significant alternative for lengthy parenteral antibiotic regimens. The many problems associated with parenteral administration of antibiotics, such as secondary infection, patient inconvenience, and high cost, are averted with oral antibiotic therapy.

Two recently developed oral antibiotics that belong to the macrolide class of antibiotics are azithromycin (an azalide) and clarithromycin (an acid stable analogue of erythromycin). These macrolides have been shown to be effective *in vitro* against a broad spectrum of bacteria and have been useful in the treatment of various kinds of bacterial infections, including methicillin sensitive *Staphylococcus aureus* osteomyelitis.^{21,29} Azithromycin gives high tissue concentrations (including bone and macrophages), low serum concentrations, and a relatively long half life.^{9,13,20,26} Such properties probably account for the observation that the *in vivo* efficacy of this drug is superior to what would be expected based on its concentrations in serum and its *in vitro* activity.²⁶ Alternatively, clarithromycin shows a high *in vitro* activity against methicillin sensitive *Staphylococcus aureus* and produces high serum concentrations, low tissue concentrations, and a shorter half life.^{9,26} Another antibiotic used in the treatment of bacterial infections is the bacterial translation inhibitor rifampin, and this agent has been identified as the most potent antibiotic against *Staphylococcus aureus*.⁸

Although these and other single agent oral therapies may represent significant hope in the treatment of methicillin sensitive *Staphylococcus aureus* osteomyelitis, their use has had only moderate effectiveness in sterilizing bone in experimental animal models. This lack of effectiveness was attributable to the inability of most oral antibiotics to achieve concentrations significantly above their respective mean inhibitory concentrations in the bone. In addition, rapid develop-

ment of resistant strains in single agent therapy has been observed in many clinical and experimental situations. Development of rifampin resistant strains rapidly occurred in experimental methicillin sensitive *Staphylococcus aureus* osteomyelitic infections.²³

Recently, various studies have shown the efficacy of combination oral antibiotic therapy. The combination of rifampin and a quinolone (ciprofloxacin or ofloxacin) often is used to treat serious *Staphylococcus* species infections to overcome the resistance problems.^{5,6} However, isolates resistant to ciprofloxacin and ofloxacin are emerging rapidly.^{4,12,15,16} Thus, it is important for investigators to evaluate potential new partner antibiotics for rifampin. When rifampin was combined with azithromycin *in vivo* in the treatment of methicillin sensitive *Staphylococcus aureus* osteomyelitis in rats, the overall activity against *Staphylococcus aureus* was increased.²⁴ This combination unites two antibiotics with high levels of tissue penetration and activity against *Staphylococcus* species.^{5,20} Thus, the combination of rifampin with azithromycin and perhaps clarithromycin should enhance the eradication of bacteria from osteomyelitic bone and prosthetic devices.

This study compared the effectiveness of oral azithromycin plus oral rifampin, oral clarithromycin plus oral rifampin, and a standard parenteral antibiotic (nafcillin) plus oral rifampin for the treatment of experimental methicillin sensitive *Staphylococcus aureus* osteomyelitis in rabbits.^{17,18,22} The study hypothesis was that macrolides may be attractive partners for rifampin for the treatment of *Staphylococcus aureus* osteomyelitis and may have comparable infection resolution rates with nafcillin plus oral rifampin. Although controls of rifampin, azithromycin, or clarithromycin alone may have been included, the authors think that previous studies of single agent oral therapy adequately have elucidated the ineffectiveness of this therapy regimen in the clinical and experimental setting.

MATERIALS AND METHODS

Organism

The strain of methicillin sensitive *Staphylococcus aureus* used in this study was obtained from a patient with osteomyelitis who was undergoing treatment at the authors' institution. The organism was stored at -70° C in defibrinated sheep's blood and was used as needed. Antibiotic tube dilution sensitivities of this *Staphylococcus aureus* strain were measured for azithromycin, clarithromycin, rifampin, and nafcillin in a cation supplemented Mueller-Hinton broth.⁷

Production of Osteomyelitis

An aliquot of this strain of *Staphylococcus aureus* was grown overnight in tryptic soy broth and diluted in 0.89% sterile saline to a concentration of 1.0×10^7 colony forming units per milliliter. New Zealand White rabbits, 6 to 8 weeks of age and weighing 1.5 to 2 kg, were used in the study. Rabbits were anesthetized using an intramuscular injection of 30 mg/kg Ketaset (Fort Dodge Laboratories, Inc, Fort Dodge, IA), 10 mg/kg Acepromazine (Fort Dodge Laboratories, Inc), and 1 mg/kg Xylazine (Rugby Laboratories, Inc, Rockville Center, NY). An 18-gauge needle was inserted percutaneously through the lateral aspect of the left tibial metaphysis into the intramedullary cavity. Five percent sodium morrhuate (0.1 mL) (Eli Lilly, Indianapolis, IN), 0.1 mL of *Staphylococcus aureus* (1.0×10^7 colony forming units/mL), and 0.1 mL of sterile saline were injected sequentially.^{17,18,22} The needle was removed, and the animal was returned to its cage.

Therapeutic Trials

The rabbits were randomized into four groups at the time of infection (Day 0), and antibiotic treatment was initiated 14 days later. Ten untreated controls that were infected (Group 1) were included. Group 2 received oral azithromycin (50 mg/kg) every 24 hours plus oral rifampin (40 mg/kg) every 12 hours dissolved in 0.5% methylcellulose.¹⁹ Group 3 received oral clarithromycin (80 mg/kg) every 12 hours plus oral rifampin every 12 hours (40 mg/kg) dissolved in 0.5% methylcellulose.¹⁹ Group 4 received subcutaneous nafcillin (40 mg/kg)¹⁷ every 6 hours plus oral rifampin every 12 hours (40 mg/kg) dissolved in 0.5% methylcellulose.¹⁹ The antibacter-

ial agents were given from Day 14 through Day 42 (28 days total). The azithromycin, clarithromycin, and rifampin were administered orally via a syringe coated with sugar,¹⁹ whereas the nafcillin was given subcutaneously into the back of the rabbits.¹⁷ After completion of the treatment regimens, the rabbits were observed for 2 weeks and sacrificed.

Radiographs of both tibias were taken at antibiotic initiation (Day 14) and at antibiotic termination (Day 42). Severity of the infection by radiographic appearance was graded by a rating system previously reported.¹⁸ The rabbits were weighed before infection and once weekly until they died or were sacrificed. The rabbits were monitored daily for stool character, caloric intake, tibial tenderness, and general health, and were weighed weekly.

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 sacrificed by an intravenous injection of sodium pentobarbital. Both tibias were removed, dissected free of all soft tissue, and processed for bacterial cultures. The bones were split into small pieces with a 5-mm, single action rongeur. The marrow was removed, and bone and marrow samples were weighed. The whole bone was pulverized in a bone mill (Retsch Bone Mill, Brinkmann Industries, Inc, Houston, TX) and suspended in 3 mL of sterile 0.85% saline per gram of bone. Tenfold serial dilutions were performed and plated on tryptic soy agar blood plate to quantitate the bacterial concentrations present. Specimens isolated from bone cultures were confirmed to be *Staphylococcus aureus* by the BBL Staphyloslide 200 Test (Becton Dickinson, Cockeysville, MD).

Drug Kinetics in Serum and Simultaneous Level Measurements in Serum and Bone

A group of animals that had not been infected and a group of animals that had been infected for 3 to 4 weeks were given a single oral dose of azithromycin (50 mg/kg body weight) dissolved in 0.5% methylcellulose solution,¹⁹ a single oral dose of clarithromycin (80 mg/kg body weight) also dissolved in 0.5% methylcellulose,¹⁹ a single oral dose of rifampin (40 mg/kg body weight) also dissolved in 0.5% methylcellulose,¹⁹ or a sin-

gle 40 mg/kg body weight subcutaneous dose of nafcillin.¹⁷ Serum concentrations for azithromycin, clarithromycin, and nafcillin were determined from blood drawn at 1, 3, 6, 12, and 24 hours after dose administration. In two other groups of animals (not infected and infected for 3 to 4 weeks), simultaneous serum and bone concentrations were determined 2 hours after a single oral dose (at the same levels as above) of azithromycin, clarithromycin, rifampin, or a single subcutaneous dose of nafcillin.

Drug Assays in Serum and Bone

An agar disc diffusion bioassay using *Sarcina lutea* (strain ATCC 9341) was used to measure all antibiotic concentrations in serum and bone eluates. The lower limits of detection for each antibiotic by these assays were determined. Antibiotic standards were made at 1000 µg/mL and diluted in 100% normal rabbit serum. Twenty microliter serum standards or serum samples were placed on blank concentration discs (0.25 inch, Difco Laboratories, Detroit, MI), 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 samples and standards, and sample concentrations were extrapolated. Blood was drawn from each rabbit before the administration of antibiotic, and if inhibitors were detected, the animal was not used in the study.

Bones (infected and not infected) were prepared for assay by dissecting them free from all soft tissue, breaking them into small pieces, and removing the marrow. The bone was pulverized in a bone mill (Janke and Kunkel, IKA Werk), weighed, and suspended in 50% 0.1 mol/L sodium phosphate buffer (pH 7.2) and 50% normal rabbit serum. Marrow also was weighed and suspended in the buffer serum solution. One milliliter of the buffer serum solution was used per 0.5 g of bone or marrow. The crushed bone or marrow with buffer serum mixture was agitated for 1 hour at 4° C. Antibiotic in the supernatant fluid was assayed by the previously described disc diffusion methods. Standard solutions of the drugs were prepared for antibiotic bone assays and marrow assays by adding either normal uninfected bone or marrow to the buffer serum solution containing known amounts of either azithromycin, clarithromycin, rifampin, or nafcillin.

RESULTS

Mean inhibitory and mean bactericidal concentration determinations: the mean inhibitory and mean bactericidal concentrations of azithromycin, clarithromycin, rifampin, and nafcillin to this strain of *Staphylococcus aureus* were 3.12 and greater than 100 mg/L, 0.39 and 12.5 mg/L, less than 0.2 and 1.56 mg/L, and 0.39 and 0.78 mg/L, respectively.

Mortality

Of the 84 rabbits infected for the study, four (4.8%) died before treatment initiation at Day 14 after infection. No animals died between treatment initiation and study termination, except those in the nafcillin plus oral rifampin treated group. Seven of the 14 animals of this group died during the treatment phase of the study because of excessive dehydration (shown by altered skin turgor) and gastrointestinal inflammation (as diagnosed by an animal facility veterinarian).

Bone Cultures

All tibias from controls that were infected but untreated ($n = 10$) revealed positive cultures for *Staphylococcus aureus* at a mean concentration of 2.8×10^4 colony forming units/g bone. The rifampin plus clarithromycin ($n = 15$) and rifampin plus azithromycin ($n = 15$) groups showed significantly lower percentages of positive *Staphylococcus aureus* infection (20% and 13.3%, respectively) and bacterial concentrations (3.5×10^1 and 1.75×10^1 colony forming units/g bone, respectively) ($p < 0.001$). The osteomyelitic tibias of the nafcillin plus rifampin treated group ($n = 7$) showed a complete obliteration of *Staphylococcus aureus* infection that also was significantly lower than that of controls ($p < 0.001$). The differences observed for bone bacterial concentrations and sterilization percentages between the treated groups were not statistically significant (Figs 1, 2).

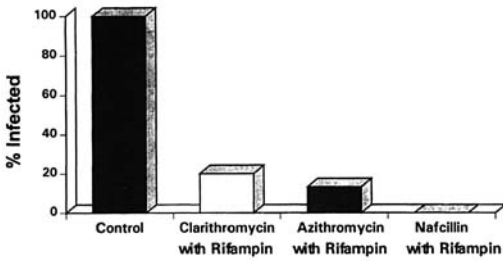


Fig 1. Percent of animals found with positive *Staphylococcus aureus* cultures detected in tibias of control (n = 10), azithromycin with rifampin (n = 15), clarithromycin with rifampin (n = 15), and nafcillin with rifampin (n = 7) groups.

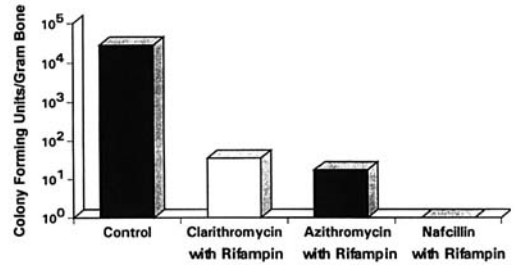


Fig 2. Mean colony forming unit levels detected in tibias of control (n = 10), azithromycin with rifampin (n = 15), clarithromycin with rifampin (n = 15), and nafcillin with rifampin (n = 7) groups.

Concentrations of Antibiotic in Serum and Bone

Concentrations of azithromycin (50 mg/kg), clarithromycin (80 mg/kg), rifampin (40 mg/kg), and nafcillin (30 mg/kg) in the sera of rabbits (infected and noninfected) after administration of the respective drugs are shown in Figure 3. The elimination of the antibiotics from the serum was fastest for nafcillin, followed by clarithromycin, rifampin, and azithromycin.

Table 1 compares simultaneous concentrations of antibiotics in serum, in bones that had not been infected, and in bones and marrow samples of infected animals 2 hours after

single dose oral antibiotic administration of azithromycin (50 mg/kg), clarithromycin (80 mg/kg), or rifampin (40 mg/kg). Data also are shown for a nafcillin group (30 mg/kg) 1 hour after subcutaneous administration. The highest antibiotic concentrations in the marrow were obtained with azithromycin and clarithromycin, with lower concentrations of these macrolides found in the bone and serum. Animals that had not been infected but treated with rifampin had low concentrations of antibiotic in the tibias. However, animals that had been infected had bone and marrow antibiotic levels approximately 10³ times greater than the levels found in animals that were not infected. However, the serum

TABLE 1. Simultaneous Serum and Bone Concentrations (µg/mL) of Azithromycin, Clarithromycin, Rifampin, and Nafcillin in Rabbits That Were or Were Not Infected

Treatment Group	Uninfected			Infected		
	Marrow	Bone	Serum	Marrow	Bone	Serum
Azithromycin	15.2	0.97	0.28	30.0	4.40	0.23
Clarithromycin	20.8	1.18	1.80	24.6	2.77	1.81
Rifampin	0.004	0.017	3.10	1.82	9.20	4.00
Nafcillin	ND	1.30	14.4	ND*	2.20	14.30

ND = not done.

Simultaneous serum and bone concentration azithromycin (50 mg/kg) (n = 6), clarithromycin (80 mg/kg) (n = 6), and rifampin (40 mg/kg) (n = 6) in rabbits that were or were not infected 2 hours after single-dose oral antibiotic administration. Data also are given for a nafcillin group (30 mg/kg) (n = 10) 1 hour after subcutaneous administration.

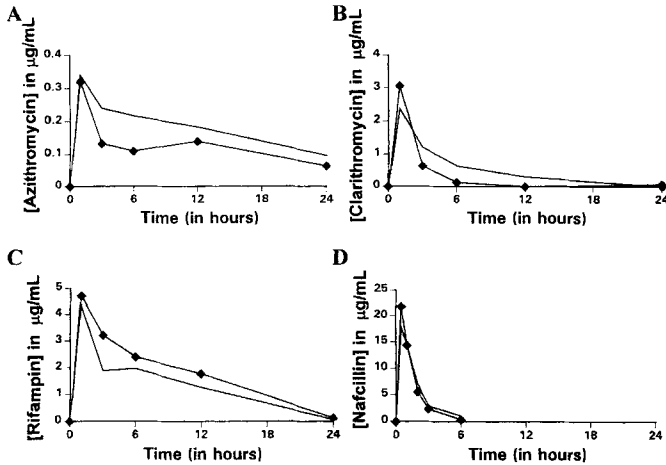


Fig 3A–D. Serum concentrations of (A) azithromycin (50 mg/kg), (B) clarithromycin (80 mg/kg), (C) rifampin (40 mg/kg), and (D) nafcillin (30 mg/kg) in animals that were (\blacklozenge) and were not infected (—) 0, 1, 3, 6, 12, and 24 hours after antibiotic administration.

rifampin concentrations in both groups were comparable and reasonably high. Nafcillin treated groups that either were infected or not infected had high and nearly identical serum antibiotic concentrations.

DISCUSSION

Clarithromycin plus rifampin, azithromycin plus rifampin, and nafcillin plus rifampin were equally effective in the treatment of *Staphylococcus aureus* osteomyelitis in rabbits. Each therapy group had bone bacterial concentrations and sterilization failures that were significantly less than those of the untreated controls ($p < 0.001$). Rabbits showed approximately 80%, 87%, and 100% tibial sterilization after treatment with clarithromycin plus rifampin, azithromycin plus rifampin, and nafcillin plus rifampin, respectively. The dosages of clarithromycin, azithromycin, nafcillin, and rifampin used in this study resulted in concentrations of antibiotics in serum similar to those achieved in humans at standard doses. All pharmacokinetic results obtained in the current study for each of the antibiotics correspond with those obtained in previous experimental studies with similar dosage regimens.^{3,5,8,9,13,17,23,24,26,27,29,30}

Azithromycin, although having low serum concentrations, produced tissue con-

centrations at 2 hours after administration that were approximately 100 times the level of that in the serum. However, this augmented tissue level may not necessarily result in drastically increased bactericidal effect in vivo because of the instability of azithromycin at the low pH usually reached in osteomyelitic bone.²⁵

The differences observed for tibial sterilization percentages and bone concentrations of viable bacteria were not statistically significant between the treated groups. The efficacy of the parenteral (nafcillin) plus oral (rifampin) treatment groups appeared to be more effective than the other groups, but the sample size ($n = 10$) was not large enough to statistically confirm this conclusion. Future studies with larger sample sizes may reveal a higher efficacy with parenterally administered antibiotics as partners for rifampin. Although no statistically significant differences were observed between the treatment groups, other characteristics of the treatment regimens were significantly different when the parenteral and oral administration methods were considered. Although nafcillin plus rifampin did not resolve experimental osteomyelitis at a significantly higher rate than did the oral therapy, nafcillin had to be administered parenterally every 6 hours (as in human treatment) and resulted in a 50% animal mortality. Seven of the 14 animals of

this group died during the treatment phase of the study because of excessive dehydration and gastrointestinal inflammation. The rabbit model of osteomyelitis is relatively susceptible to the toxic effects of antibiotic therapy. High dose long-term therapy with some antibiotics, including the penicillins, cephalosporins, and clindamycin, has been associated with significant rabbit mortality, probably related to idiopathic diarrhea or pseudomembranous colitis.¹⁴

In this study, oral antibiotic therapy was equally as effective as parenteral antibiotic therapy in eradicating experimental *Staphylococcus aureus* osteomyelitis. Parenteral antibiotic therapy often requires hospital admittance of the patient for the duration of the antibiotic treatment to assure correct administration of the antibiotic(s). The extreme cost and patient inconvenience associated with a 4- to 6-week hospital stay have led to the use of outpatient intravenous therapy using long term intravenous access catheters, such as Hickman or Groshong catheters.^{2,10,11} However, these catheter systems may produce catheter sepsis, localized infection, pneumothorax, hemothorax, and accidental air embolism.

Effective oral antibiotic treatment represents a significant development in the treatment of osteomyelitis. Some problems associated with oral antibiotic therapy may include patient compliance, gastrointestinal intolerance, and reduced levels of serum and tissue perfusion with some antibiotics. Thus, it is up to every treating physician to judge whether the patient will be compliant, will be able to complete the standard oral therapy independently for the prescribed time, will tolerate the antibiotic regimen, and will have appropriate tissue and serum antibiotic penetration. However, even if the patient is not compliant, one still should consider prescribing oral dosing in the hospital, monitored outpatient oral dosing at the hospital, or monitored oral dosing in the home. Although the recommended treatment of osteomyelitis is operative debridement and parenteral ad-

ministration of antibiotics for 4 to 6 weeks, the many benefits associated with oral antibiotic treatment should warrant a concentrated look at this treatment modality.

Combination oral antibiotic therapy recently has relied on the pairing of rifampin with the quinolone class of antibiotics (ciprofloxacin or ofloxacin). Because of the recent development of a large number of ciprofloxacin and ofloxacin resistant Gram positive organisms, a new partner for rifampin must be found. Although macrolides traditionally are not used in the treatment of osteomyelitis, these reported results indicate that azithromycin and clarithromycin may be attractive partners for rifampin for the treatment of *Staphylococcus aureus* osteomyelitis.

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