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Comparative Efficacy of Rifabutin and Gatifloxacin in the Rat Osteomyelitis Model

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Abstract

Background: Osteomyelitis is a difficult-to-treat infection requiring prolonged antibiotherapy. *Staphylococcus aureus* is the most common organism isolated from bone infections. Gatifloxacin (GAT) and rifabutin (RFB) are highly active against *S. aureus in vitro*. Here, we used different models of therapy to compare the efficacy of GAT and RFB in a model of rat osteomyelitis. **Methods:** Osteomyelitis was established in CD rats (200-225 g) by injecting 0.05 mL of 5% sodium morrhuate followed by $2x10^7$ cells of *S. aureus* ATCC13709 (minimum inhibitory concentrations: GAT, 0.12 µg/mL; RFB, 0.016 µg/mL) into the medullary cavity of the tibia. Three models of therapy were used:
1) Prophylaxis model (n=5/group): Rats received an intravenous dose of GAT (10 mg/kg) or a subcutaneous (SC) dose of RFB (20 mg/kg) 2 days prior to infection. Tibiae were harvested 1 day post-infection (PI) for bacterial counts. 2) Treatment model (n=10/group): Therapy started 14 days PI and lasted for 21 days. Infected rats received a daily SC dose of RFB at 20 mg/kg or twice daily oral dose of GAT at 40 mg/kg. Bacterial counts in tibia were assessed 24 h after the last dose. 3) Dose Ranging model (n=5/dose): Therapy started 14 days PI and lasted for 7 days. GAT and RFB were injected SC once daily at 5 to 80 and 0.1 to 40 mg/kg, respectively. The bacterial counts in bone were assessed 24 h after the last dose. Dose-response data were analyzed by a sigmoïd Emax model. **Results:** 1) No bacteria were detected (< 2 Log CFU/g) from all rats treated with RFB while those treated with GAT remained infected (6.1±0.50 Log CFU/g), as found in the untreated group (6.0±1.1 Log CFU/g). 2) In the treatment model, 6.7±0.26 Log CFU/g were recovered from untreated rats, and 2.1±0.14 (80% of tibia < 2 Log CFU/g) and 2.4±0.41 Log CFU/g (40% of tibia < 2 Log CFU/g) from rats treated with RFB and GAT, respectively. 3) E_{max} value for RFB was 5 mg/kg and 60 mg/kg for GAT. Calculated ED_{50} was 2.0±0.36 mg/kg for RFB and 38±3.6 mg/kg for GAT. **Conclusion:** Both GAT and RFB showed efficacy in the rat osteomyelitis model. However, in all 3 therapy models, RFB was more potent than GAT.

Introduction

Osteomyelitis is a difficult-to-treat bone infection which usually involves a prolonged course of antibiotic therapy, surgical interventions and significant morbidity[1]. No antibacterial agent is currently approved by the FDA to treat osteomyelitis; surgical debridement and 4 to 6 weeks of therapy are considered to be the gold standard for treatment[2]. *Staphylococcus aureus* is the most common organism isolated from osteomyelitis patients[2] and methicillin-resistant *S. aureus* (MRSA) emergence is increasingly frequent[3]. Novel, potent antibacterial agents are needed to counteract treatment failures and drug-resistant organisms. Rifamycins are highly potent against *S. aureus*, including MRSA, with documented activity against biofilms *in vivo* [4] and intracellular activity *in vitro* in a culture of infected monocytes[5]. Gatifloxacin (GAT) is a 8-methoxy fluoroquinolone with potency against gram-positive cocci, including multiple-drug resistant strains.[6] The mechanism of action of GAT relies on inhibition of bacterial DNA gyrase, while rifabutin (RFB) inhibits bacterial RNA polymerase[7]. GAT therapy was reported to be effective in eradicating experimental *S. aureus*-induced osteomyelitis in a rabbit model[8]. The rat osteomyelitis model has been widely used for determination of efficacy of experimental chemotherapy[1]. Here, we used different models of therapy to compare the efficacy of GAT and RFB in rat models of both acute and chronic *S. aureus* osteomyelitis.

Methods

***In vitro* activities:** Minimum inhibitory concentrations (MICs) were determined by CLSI broth microdilution against methicillin-sensitive *S. aureus* ATCC 13709. MICs for GAT and RFB were 0.12 and 0.016 µg/mL, respectively.

Animal studies: All studies were performed in accordance with protocols that were approved by the Institutional Animal Care and Use Committee. Osteomyelitis was established in CD rats (200-225 g) as described by O'Reilly and Mader[9]. Briefly, rats were anesthetized with isofluorane gas and a primary incision was made over the tibia region. The tibia was exposed and a hole was drilled into the bone. The infection was established by injecting 0.05 mL of 5% sodium morrhuate followed by $2x10^7$ cells of *S. aureus* ATCC13709 into the medullary cavity of the tibia.

After bacterial inoculation, the hole was sealed with dental gypsum and the wound was closed with metal clips. At the experimental endpoints, rats were euthanized by CO_2 and tibiae were harvested, weighed and ground to powder. Bone powder was resuspended in 5 mL PBS in the presence of charcoal (so as to limit antibiotic carryover), serially diluted and plated on tryptic soy agar plates for bacterial counting. The bacterial load was expressed as colony forming units (CFU)/g of wet tibia bone (Log CFU/g). The limit of detection was 2 Log CFU/g of tibia bone. For the calculation of the mean, 1.9 Log CFU/g were used when no bacteria were detected.

Statistical calculations: The statistical calculations were performed according to the Kruskal-Wallis test, the Mann-Whitney U test and/or the unpaired t test by using StatsDirect software. P-values below 0.05 were considered significant.

Prophylaxis model: Antibiotics were administered as a single dose 2 days before infection. Rats (n=5/group) were split in the following treatment groups: no treatment, an intravenous injection of GAT at 10 mg/kg; or a subcutaneous injection of RFB at 20 mg/kg. A group of rats received 10 mg/kg of moxifloxacin intravenously 1h after infection as a positive control group. Animals were euthanized 24h post-infection.

Treatment model: The therapy was started 14 days post-infection and lasted for 21 days. Rats (n=10/group) received no treatment, a subcutaneous injection of RFB at 20 mg/kg daily, or an oral dose of GAT at either 6 or 40 mg/kg twice a day. A group of rats received a daily dose of rifampicin subcutaneously at 20 mg/kg as a positive control group. Rats were euthanized 24h after the last day of treatment.

Dose ranging model: The therapy started 14 days post-infection and lasted for 7 days. Rats (n=5/group) received either no treatment, or a daily subcutaneous injection of GAT or RFB. Tested doses ranged from 5 to 80 mg/kg for GAT and 0.1 to 40 mg/kg and RFB. Rats were euthanized 24h after the last dose. The dose response data were analyzed by a sigmoïd E_{max} model.

Results

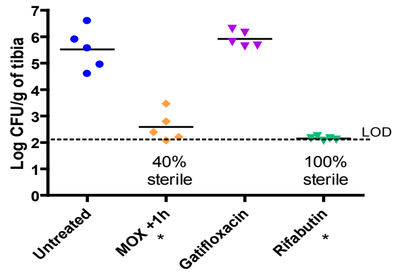

Figure 1. Efficacy of gatifloxacin and rifabutin when administered 2 days prior to the infection as a prophylactic treatment.
MOX +1h, moxifloxacin given 1h after infection; Limit of detection (LOD) ≤ 2 Log CFU; * p ≤ 0.05

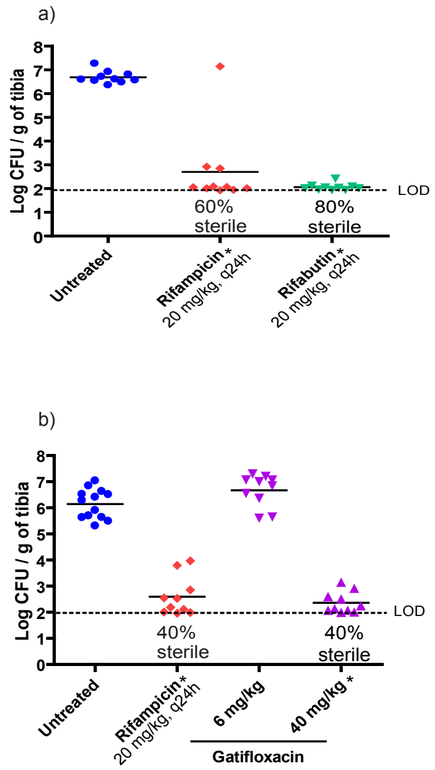

Figure 2. Efficacy of a) rifabutin and b) gatifloxacin administered for 21 days in the rat osteomyelitis model.
Limit of detection (LOD) ≤ 2 Log CFU; * p ≤ 0.05

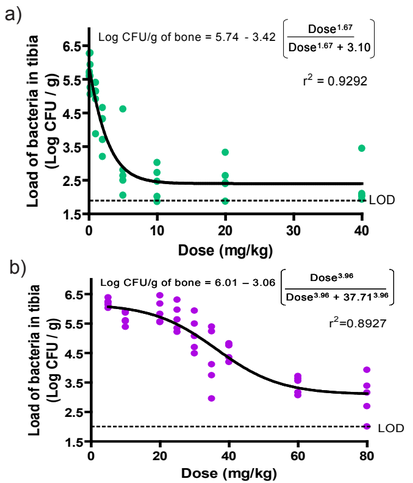

Figure 3. Dose ranging study of a) rifabutin and b) gatifloxacin administered for 7 days in the rat osteomyelitis model.
Limit of detection (LOD) ≤ 2 Log CFU.

Table 1. Efficacy of gatifloxacin and rifabutin in the rat chronic osteomyelitis models of treatment.

		Efficacy		
		21 days treatment	7 days treatment	
Drug	Dose (mg/kg)	Δ Log CFU/g tibia	ED_{50} (mg/kg)	E_{max} (mg/kg)
Gatifloxacin	40	-3.6 (40% = LOD)	38 ± 3.6	60
Rifabutin	20	-4.6 (80% = LOD)	2.0 ± 0.4	5

Conclusions

§ Prophylaxis model:

- RFB showed superior efficacy to GAT as a prophylactic treatment
- This finding correlated well with the superior *in vitro* activity of rifabutin (MIC = 0.016 µg/mL) and its long *in vivo* half-life (36-67 h)[10, 11]

§ Treatment model:

- The efficacy of GAT was dose-dependent
- GAT was highly active when administered at 40 mg/kg twice a day
- Treatment with rifabutin yielded superior efficacy relative to GAT

§ Dose ranging model:

- Efficacies of RFB and GAT were dose-dependent
- RFB and GAT yielded an ED_{50} (dose resulting in 50% of the maximal killing) of 2.0 ± 0.4 mg/kg and 38 ± 3.6, respectively, as calculated using the E_{max} model

The efficacy data obtained for RFB and GAT support the idea that they could represent attractive antimicrobial agents for the prevention (rifabutin) and the treatment of osteomyelitis caused by *S. aureus*.

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