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Comparative activities of vancomycin, tigecycline and rifampin in a rat model of methicillin-resistant *Staphylococcus aureus* osteomyelitis

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Summary *Objectives:* Implant-associated methicillin-resistant *Staphylococcus aureus* (MRSA) infections are challenging to treat. We compared antimicrobial activities in a rat model of chronic osteomyelitis in the context of retention of the foreign body without débridement.
Methods: MRSA was inoculated into the proximal tibia and a wire implanted. Four weeks after infection, treatment with vancomycin 50 mg/kg every 12 h, tigecycline 14 mg/kg every 12 h, rifampin 25 mg/kg every 12 h, or the combination of vancomycin or tigecycline plus rifampin was administered intraperitoneally for 21 days.
Results: MRSA was cultured from all tibias in the control group (median, 6.06 $\log_{10}$ CFU/g bone). Median bacterial counts ($\log_{10}$ CFU/g) at 48 h post-treatment were 6.16 for vancomycin ($p = 0.753$), 2.29 for vancomycin plus rifampin ($p < 0.001$), 5.90 for tigecycline ($p = 0.270$), 0.10 for tigecycline plus rifampin ($p < 0.001$), and 0.91 for rifampin ($p = 0.044$) treatment. Three deaths were observed in the tigecycline plus rifampin group. Median bacterial counts ($\log_{10}$ CFU/g) at two weeks post-treatment were 5.65 for vancomycin ($p = 0.6$), 4.05 for vancomycin plus rifampin ($p = 0.105$), 5.68 for tigecycline ($p = 0.401$), 4.05 for tigecycline plus rifampin ($p = 0.028$), and 5.98 for rifampin ($p = 0.297$) treatment.

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Conclusions: Tigecycline plus rifampin resulted in a significant bacterial count decrease, an effect more prominent at 48 h than two weeks after treatment completion. Tigecycline was not well tolerated at the dose studied.

Introduction

Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) are a major public health concern and their treatment can be challenging. In a meta-analysis of 22 studies, vancomycin minimum inhibitory concentration (MIC) values of 1.5 μg/ml or higher were predictive of treatment failure, irrespective of infection source.[1] Further data on clinical outcomes with the use of alternative agents are required; animal models aid in the evaluation of those agents.

Tigecycline is a bacteriostatic glycylcycline antibiotic that inhibits protein synthesis by binding to the 30S ribosomal subunit and preventing amino acids from becoming incorporated into elongating peptide chains. The tigecycline bone to serum AUC_{0-24h} ratio was 0.41 among 25 adult human subjects after a single 100-mg dose.[2] It has been approved by the United States Food and Drug Administration (FDA) for use in skin and soft tissue infections, complicated intraabdominal infections, and community-acquired pneumonia, but not osteoarticular infections.

Minocycline or doxycycline is recommended by the Infectious Disease Society of America as a secondary companion drug in combination with rifampin for the treatment of prosthetic joint infection after completion of pathogen-specific intravenous antimicrobial treatment.[3] Their use is also recommended for chronic oral antimicrobial suppression. Similar recommendations are included in the French[4] and Italian guidelines.[5] The role of tigecycline is not addressed in these guidelines.

Tigecycline has been shown to be effective in animal models of osteomyelitis, in the absence of a foreign body.[6,7] The presence of an implant renders such infections more difficult to treat, due to the existence of bacteria in biofilms, protecting the bacteria from killing by innate host defenses and antimicrobial agents. In clinical practice, select foreign body-associated orthopedic staphylococcal infections may be managed with implant retention, involving surgical débridement in combination with antimicrobial treatment. To the best of our knowledge, tigecycline has not been evaluated for treatment of implant-associated osteoarticular infection. The objective of the present study was to assess the activity of vancomycin, tigecycline, rifampin and combinations thereof in a rat model of chronic foreign body MRSA osteomyelitis.

Materials and methods

Microorganism and antimicrobial agents

MRSA IDRL-6169, recovered from a patient with a prosthetic joint infection, was studied. Vancomycin was obtained from Hospira Inc., Lake Forest, IL. Tigecycline was obtained from Pfizer Inc., New York, NY. Rifampin was obtained from Akorn Strides, Lake Forest, IL. The MIC of each antimicrobial agent was determined by broth microdilution using an inoculum of $5 \cdot 10^5$ colony forming units (CFU)/ml according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[8] In order to minimize oxidative degradation of tigecycline, Mueller-Hinton broth prepared within 12 h was used for MIC determination. The minimum bactericidal concentration (MBC) was determined by broth microdilution according to CLSI guidelines.[9]

Pharmacokinetic studies

Tigecycline was administered intraperitoneally to 15 healthy male Wistar rats. After a loading dose of twice the maintenance dose, tigecycline was administered at 7 mg/kg or 14 mg/kg every 12 h. These doses were used in previous animal experiments[6,7] and were chosen to approximate human pharmacokinetics. Serum concentrations were measured on day 3 at 0.5, 1, 2, 4, 8, and 12 h after the last dose. Blood was collected by cardiac puncture or tail vein bleed at each time point and serum was separated by centrifugation. Samples were stored at −70 °C. Antimicrobial concentrations were determined by high-performance liquid chromatography. Blank rat serum was used as a control. Pharmacokinetic parameters were calculated using PK solutions 2.0 (Summit Research Services, Montrose, CO).

Experimental rat model

Experimental chronic foreign body osteomyelitis was established in male Wistar rats (weighing 250—350 g), as previously described.[10] Briefly, general anesthesia was induced by intraperitoneal administration of ketamine (60 mg/kg) and xylazine (6 mg/kg). The proximal third of the left tibia was surgically exposed, and a 1.5-mm hole drilled into the medullary cavity. Fifty microliters of 10^6 CFU suspension of the MRSA isolate was injected into the bone. Based on previous experiments using this inoculum, we are able to establish bone infection without causing sepsis or animal death. Subsequently, a 5 mm × 1 mm stainless steel wire (Zimmer, Warsaw, IN) was implanted into the bone. The hole was covered with dental gypsum. The skin was closed with tissue glue and surgical clips. The wound was sprayed with antiseptic. Buprenorphine (0.05 mg/kg) was administered subcutanesously for analgesia. The study was approved by the Institutional Animal Care and Use Committee of the Mayo Clinic.

Four weeks after establishing infection, animals were arbitrarily assigned to one of six study groups, each consisting of 16 animals: No treatment (control), vancomycin monotherapy, vancomycin plus rifampin, tigecycline monotherapy, tigecycline plus rifampin, and rifampin monotherapy. Vancomycin was administered at 50 mg/kg

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