Objective: Radezolid (RZD) is an investigational oxazolidinone with excellent in vitro and in vivo activity against a variety of Gram-positive bacteria including methicillin-resistant Staphylococcus aureus (MRSA). RZD has been shown to fit the finding that nascent accumulation in vivo in macrophages propel its pharmacokinetic advantage over linezolid, ampicillin, and cephalosporins. These findings have not been observed for linezolid.

Methods: RZD is a highly water-soluble drug. Pharmacokinetic Analysis was determined in a 3-week post drug administration using an LC/MS/MS with a Waters Alliance HT 2795 LC system was used. Bioanalytical methods were performed in accordance with Rib-X's Institutional Animal Care and Use Committee.

INTRODUCTION

Radezolid (RZD), a novel oxazolidinone discovered by Rib-X Pharmaceuticals Inc. using its proprietary structure-based drug design approach, is a highly active, orally absorbed, clinically relevant drug resistant to MRSA and other Gram-positive agents such as vancomycin and teicoplanin, with negative and antagonist synergism. Radezolid has successfully completed Phase 2 trials in uninfected and skin structure and community-acquired pneumonia.

Ethics: Efficacy has been observed in humans and animals against various types of infections in spite of high protein binding (>60% across all species). Therefore, investigations have been undertaken to elucidate the mechanisms of activity. Lematine et al. [2] have shown that RZD has been found to accumulate in various tissue types in vivo including muscle, skin, and bone. This accumulation correlated with intracellular active metabolites in vivo in vivo.

In addition, studies have shown that radezolid has increased potency against intracellular S. aureus compared to that of linezolid. Since tissue concentrations of drug, in particular at the site(s) of infection, are critical for bacterial eradication, Rib-X has undertaken studies further assess the impact of intracellular accumulation of radezolid. This ability to localize within tissues likely improve radezolid efficacy due to the carriage of drug to infection sites via host-defense cell recruitment.

METHODS

Bacteria, animals and infection procedure. Bacteria (Staphylococcus aureus ATCC29213, subsp. aureus Smith or St. aureus USA800) were prepared for infection by inactivating from frozen stock onto Triple Tsay agar containing 5% sheep blood (Becton Dickinson, 24246). Isolated colonies were inoculated into 3 ml of Mueller-Hinton broth (Becton Dickinson and Company) and were incubated at 35°C for 24 hr. The cultures were grown overnight in a roller drum incubator at 150 rpm, after which each OD600 was measured. The cultures were grown over a period of 2-3 days until they reached an OD600 of 0.7-0.8. Furthermore, the cultures were grown in an anaerobic chamber for 1-2 h at 37°C, with shaking. The preparation corresponds to 1-5 x 10^9 colony-forming units per ml. For all studies, eighth to twelve week old female BALB-C (Charles River Laboratories) weighing 25-30 g were used. In some experiments, mice were rendered neutropenic by an intraperitoneal injection of cyclophosphamide (Sigma) four days and one day prior to infection (150 and 100 mg/kg, respectively). Mice were injected into both caudal thigh muscles. Mice were returned to their cages and allowed food and water ad libitum.

For all studies, eight-to-nine week old female ICR (CD-1, Charles River Laboratories) weighing 25-29 g were used. In some experiments, mice were neutropenic by an intraperitoneal injection of cyclophosphamide (Sigma) four days and one day prior to infection (150 and 100 mg/kg, respectively). Mice were injected into both caudal thigh muscles. Mice were returned to their cages and allowed food and water ad libitum.

Lematine et al. [2] have shown that RZD has been found to accumulate in various tissue types in vivo including muscle, skin, and bone. This accumulation correlated with intracellular active metabolites in vivo in vivo. In addition, studies have shown that radezolid has increased potency against intracellular S. aureus compared to that of linezolid.

All animal studies were performed in accordance with NIH's Institutional Animal Care and Use Committee.

Bioanalytical methods. Aerobic strains were used for protein precipitation of the samples and the human homolog type. A Quortex Micro mass spectrometer was used for HPLC analysis with electrospray positive ionization in the multiple reaction monitoring mode. Mass spectrometry for both CD and GQ were set at a mass range. Dwell times were set to 1.5 sec. Collision energy was used typically. The X Waters Alliance HT 2755 LC system was equipped with a reversed-phase YMC 100A column for chromatographic separation. The linear dynamic range of the LC/MS/MS method was 1-10 nM.

Pharmacokinetic Analysis. Pharmacokinetic parameters were determined for each simultaneously administered dose using GraphPad WinNonlin. 6.2.