In Vitro Activity of LY333328, a New Glycopeptide, against Extracellular and Intracellular Vancomycin-Resistant Enterococci

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Summary
The objectives of the study were to observe the activity of LY333328, a new semisynthetic glycopeptide, compared to that of vancomycin against six strains of Enterococcus faecium and Enterococcus faecalis, including four vancomycin-resistant strains. Bacteria ingested by polymorphonuclear leukocytes (PMN) as well as extracellular bacteria were studied using a colony count method. The activity against intracellular bacteria was tested with the drugs present in the extracellular medium, as well as after preincubating the PMN and removal of the drugs. LY333328 is active against the tested enterococci, regardless of their susceptibility to vancomycin, with MICs of 1–2 mg/l. It is bacteriostatic against extracellular enterococci at concentrations of 2 µg/ml and above regardless of their resistance to vancomycin. After 4 h incubation at 10 MIC, vancomycin-resistant strains of E. faecium and E. faecalis located intracellularly were reduced by 55% and 90%, respectively. Even after preincubation and removal of the drug, LY333328 had an effect at 10 MIC with a 20–30% reduction in the inoculum. The results suggest that in contrast to vancomycin, LY333328 is active against intracellular vancomycin-resistant enterococci, particularly E. faecalis, even after removal of the extracellular drug.

Key Words
LY333328 · In vitro Activity · Enterococci · VRE · Vancomycin

Introduction
In a U.S. nation-wide study, 4.4% of the enterococci found in blood cultures were resistant to vancomycin [1]. In a European multicenter study carried out in 1995, no Enterococcus faecalis isolates and 3.8% of Enterococcus faecium isolates were found to be resistant to vancomycin or teicoplanin [2]. The increasing prevalence of vancomycin-resistant enterococci (VRE) is one of the major challenges of anti-infective research. There is no standard therapy for infections with VRE. Although some agents have shown in vitro activity, including aminoglycosides, ciprofloxacin, doxycycline, teicoplanin, chloramphenicol and rifampin, treatment options are limited to combinations of drugs with marginal in vivo efficacy against the pathogens [3]. A new therapy under investigation is the combination of quinupristin and dalfopristin, which is reported to be effective against infections with VRE [3].

LY333328 is a new semisynthetic glycopeptide, an N-alkylated derivative of a naturally occurring vancomycin analog (LY264826) [4]. It is reported to be active in vitro against many resistant gram-positive strains including methicillin-resistant Staphylococcus aureus (MRSA) and VRE [5]. Against vancomycin-resistant E. faecium, LY333328 was shown to be at least as active as vancomycin [6].

Phagocytosis of pathogens by polymorphonuclear cells (PMN) plays a major role in the defense against infections. As some pathogens are shown to be intracellular, persistent intracellular penetration as well as activity of the drug are desirable to prevent recurrent infection or failure of therapy [7]. The available pharmacokinetic data for LY333328 show unique pharmacokinetic properties with a slow elimination rate resulting in a terminal half-life of 10.5 days [8]. Currently no data on intracellular concentration or accumulation of LY333328 are available. Besides testing its activity against extracellular enterococci and enterococci ingested by human phagocytes (PMN), it is of interest to determine whether preincubation of the PMN with the drug results in intracellular accumulation leading to enhanced killing of ingested enterococci.

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Materials and Methods
Bacterial Strains
Six strains of enterococci were studied: two quality-control strains (QC), *E. faecium* 6571 and *E. faecalis* ATCC 29212; four VRE comprising two *E. faecium* and two *E. faecalis* isolates. One of each was a recent clinical isolate from hospitalized patients. The other two were laboratory strains (kindly donated by P. Courvalin, Paris): *E. faecium* BM4147 with VanA genotype and *E. faecalis* VS 83 specified as VanB. All strains were preserved weekly on fresh Mueller-Hinton agar. The bacterial suspensions were prepared as overnight cultures in Mueller-Hinton broth at 37 °C. Concentration was turbidimetrically controlled. For opsonization 107 bacteria/ml were incubated with 10% pooled autologous serum for 15 min.

Drugs and Media
Vancomycin and LY333328 were kindly provided by E. Lilly, Germany. The drugs were freshly prepared for each experiment. All experiments were carried out in medium 199 (Merck, no. F0615). The sensitivity of the strains to the antibiotics was assessed by minimum inhibitory concentration (MIC) determination in Mueller-Hintonbroth.

PMN
Cells were harvested from fresh blood of healthy male volunteers less than 30 years of age. PMN were enriched by differential centrifugation on a Percoll density gradient. Viability was checked.