Pharmacodynamics of linezolid in a clinical isolate of Streptococcus pneumoniae genetically modified to express lux genes

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A bioluminescent clinical isolate of Streptococcus pneumoniae was used to test the real-time effects of the oxazolididnone antibiotic, linezolid, on metabolism compared with effects on cell replication. Viable counts and bioluminescence measurements showed that linezolid has little bactericidal effect, which was similar at minimum (6 mg/L), intermediate (13 mg/L) and maximum (20 mg/L) serum concentrations. The post-antibiotic effect, however, was shorter when measured by light output than by viable counts. The results demonstrate that bioluminescence provides a rapid and sensitive means of measuring the effect of antimicrobials on bacterial metabolism, and that the latter recovers earlier than commencement of cell replication after linezolid exposure.

Keywords: bioluminescence, Streptococcus pneumoniae, oxazolidinones

Introduction

Streptococcus pneumoniae is the most frequent bacterial cause of pneumonia, acute meningitis and otitis media in children and adults. Management of pneumococcal infections is complicated by the emergence worldwide of penicillin and multi-antibiotic resistance among S. pneumoniae isolates, which is mainly caused by the unnecessary use of antibiotics.1 As a result, there is an urgent need for new agents with good activity against S. pneumoniae and other drug-resistant bacterial pathogens.

Linezolid is the first of a new class of synthetic antimicrobial agents, oxazolidinones, to be approved for clinical use against many resistant Gram-positive pathogens.2 It has a potent range of activity against multiresistant staphylococci, enterococci and streptococci. Oxazolidinones disrupt bacterial growth by inhibiting the initiation process of protein synthesis. Specifically, they block the formation of the 70S initiation complex by binding to the 50S ribosomal subunit.3 This site of inhibition is different from those of other protein synthesis inhibitors that interfere with the translation elongation process; hence, cross-resistance with other protein synthesis inhibitors has not been reported.4 Linezolid, which has little activity against Gram-negative bacteria, is known to be bacteriostatic against staphylococci and enterococci, and has a modest bactericidal effect on most streptococci.5

Microorganisms expressing the lux operon are able to emit light, as a result of the activity of bacterial luciferase. This activity involves the oxidation, by molecular oxygen, of reduced flavin mono-nucleotide (FMNH2) and a long-chain aldehyde to produce FMN, acid and blue–green light. Since FMNH2 production depends upon functional electron transport, only metabolically active cells can produce light. Bioluminescence is an extremely sensitive, non-destructive, real-time reporter of cell metabolism that has been used successfully to monitor the effect of antimicrobials.6,7 In this study, we used bioluminescence to investigate the pharmacodynamics of linezolid on a clinical isolate of S. pneumoniae, modified to express the lux operon. We compared the method with that of traditional colony counting. The transformed strain retained its ability to form capsules and demonstrated growth and antimicrobial susceptibility identical to the parent strain.7

Materials and methods

Test organism, antibiotics and media

S. pneumoniae SMH 11662/pAL2, a clinical isolate from Southmead Hospital (Bristol, UK), was transformed with pAL2 plasmid containing lux ABCDE operon from Photorhabdus luminescens.7 This isolate emits light constitutively and stably at 37°C and thus is an accurate reporter of cellular metabolic activity. The strain was maintained on blood agar supplemented with erythromycin (150 mg/L). Studies on S. pneumoniae were performed in brain–heart infusion (BHI) broth (Oxoid, Basingstoke, UK). Linezolid (a gift from Pharmacia Corporation, Peapack, NJ, USA) was stored and prepared according to the manufacturer’s guidelines. The MIC of linezolid for S. pneumoniae SMH 11622/pAL2 was determined by a broth macrodilution method.8

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Time–kill measurements

The activity of linezolid against *S. pneumoniae* expressing the lux operon (MIC, 1.0 mg/L) was determined by monitoring bioluminescence and viable colony counts in static cultures at 37°C. Linezolid was added at concentrations of 6 mg/L (minimum concentration in serum, C_{min}), 13 mg/L (intermediate concentration, C_{int}) and 20 mg/L (maximum concentration in serum, C_{max}). At intervals, bioluminescence was measured in an automated bioluminometer–photometer (Lucy1, Anthos, Salzburg, Austria), and viable counts were determined by plating cells onto blood agar plates with a spiral plater (Autoplate model 3000, Spiral Biotech, Maryland, USA).

Measurement of bacterial regrowth

Log-phase cultures of *S. pneumoniae* SMH 11622/pAL2 were incubated with linezolid (6, 13 and 20 mg/L) for 1 h at 37°C. Light output and viable counts were determined before and after the addition of antibiotic. After incubation for 1 h, the cells were immediately spun down, washed and diluted (1:10) in fresh, pre-warmed BHI broth and allowed to recover at 37°C. Samples were taken to measure bioluminescence and viable counts over a 24 h period. A control culture was prepared and treated identically to the test cultures, but without exposure to linezolid. The post-antibiotic effect (PAE) was obtained from the regrowth curves by calculating the difference in time taken by experimental and control cultures to increase 1 log_{10} above the count observed immediately after drug removal. The control effective regrowth time (CERT) was calculated similarly, as the difference in time required for the bacteria to resume logarithmic growth and return to the pre-exposure inoculum.

Results

Concentration-independent killing

Figure 1 depicts the killing rates of three different concentrations of linezolid against *S. pneumoniae* SMH 11622/pAL2, as determined by monitoring viable plate counts (cfu/mL) and bioluminescence (relative light units, RLU). The kill curves show that linezolid produced only minimal bactericidal activity (1 log_{10} decrease over 6.5 h) when measured by either method. In addition, independent of concentration, linezolid inhibited bioluminescence and viable counts.

Bacterial recovery after exposure to linezolid

The regrowth of *S. pneumoniae* SMH 11622/pAL2 culture after treatment for 1 h with linezolid was monitored by viable counts and bioluminescence (Figure 2). Bacterial recovery was similar following exposure to the various drug concentrations. However, the recovery rate was modified by the method used, and commenced 1 h after drug exposure when monitored by bioluminescence and 4 h after drug exposure when measured by viable counts. Moreover, the PAE of linezolid at 6, 13, and 20 mg/L, when measured by colony counts, was considerably longer (4.3, 4.6 and 4.7 h) than the PAE determined by bioluminescence (1.7, 1.9 and 2.3 h) of the same culture. The calculated CERT values of 5.0, 5.4 and 5.5 h based on viable counts were also slightly longer than those for bioluminescence (4.4, 5.1 and 5.3 h).

Discussion

In this work, we have demonstrated that light output from a self-bioluminescent Gram-positive pathogen can be used for rapid and real-time evaluation of antimicrobial pharmacodynamics. Kill curve data showed that during 20 h in static broth culture, the effect of linezolid on both bioluminescence and colony counts of *S. pneumoniae* was similar and concentration-independent. Cultures exhibited a slow exponential decrease, indicating that the bactericidal effect of linezolid was minimal. Similar results have been reported by Zarenko et al. However, the PAE of linezolid was considerably shorter when determined by light output than when determined by the traditional viable count method. This is in contrast to previous work where direct methods have given either longer, or very similar PAEs compared to viable counts. The shorter PAEs of viable counts are thought to be the result of long chain or spheroplast formation, which have been shown elsewhere to give falsely low post-exposure viable counts. Here the viable counts and bioluminescence show a very similar post-exposure drop (Figure 2), possibly because of the limited bactericidal action of linezolid. The observed reduced PAE of linezolid, when measured by direct methods, indicates that recovery of cellular metabolism (as measured by bioluminescence) is more rapid than that of cell replication (as determined by colony plate count). There is an absence of data from other direct methods monitoring metabolic activity, but our findings are in accordance with those from animal models, which show little or no PAEs of linezolid in *S. pneumoniae*.

It is interesting that CERT, which has previously been found to be method-independent, is also slightly longer when measured by viable counts than by bioluminescence monitoring. This supports the

![Figure 1](https://example.com/)
Bioluminescent *S. pneumoniae* to study linezolid effects

view that following exposure to linezolid, metabolic activity may recover more rapidly than cell replication.

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References