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Evaluation of telithromycin against *Streptococcus pneumoniae* with ribosomal mutations utilizing in vitro time–kill methodology

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Abstract

In a recent study, our in vivo data suggested that clinically achievable levels of telithromycin are more effective than azithromycin against selected *Streptococcus pneumoniae* isolates with ribosomal mutations in 23S rRNA gene alleles and L22 region mutations. In the current study, we attempt to investigate further the antibacterial activity of telithromycin against these isolates to better delineate the disparity between isolates based on allelic differences. Four isolates of *S. pneumoniae* with ribosomal mutations were tested using in vitro time–kill methodology. Isolates were exposed to telithromycin at concentrations of 0.5–8 × the minimum inhibitory concentration (MIC). At these exposures, telithromycin demonstrated concentration-dependent killing for three of the four isolates. Against the fourth isolate, telithromycin affected only a 1 log decrease in colony-forming units/mL despite exposures of 8 × MIC. These data demonstrate the in vitro killing profile of telithromycin against *S. pneumoniae* isolates with ribosomal and L22 mutations. Whilst telithromycin did not demonstrate bactericidal activity against all isolates in these time–kill studies, the in vivo human-simulated exposures did result in a high degree of bacterial kill. Full evaluation of the potential utility of new antimicrobial agents against these emerging genotypic profiles requires both in vitro and in vivo assessments.

Keywords: Telithromycin; *Streptococcus pneumoniae*; Bactericidal activity; Time–kill method

1. Introduction

Mutations in *Streptococcus pneumoniae* confer resistance to a wide variety of antimicrobial agents. In particular, there has been a growing concern over the steady rise in resistance of *S. pneumoniae* to macrolides. Whilst this resistance has been primarily attributed to active efflux (*mef*-mediated resistance) or to dimethylation of the drug binding site (*erm*-mediated resistance), alterations in genes encoding the 23S rRNA and L4 and L22 riboproteins have been described [1]. Telithromycin, the first commercially available ketolide agent, remained active against these isolates with minimum inhibitory concentrations (MICs) ≤0.25 μg/mL, well below the susceptibility breakpoint of 1.0 μg/mL.

Recent work by our group described the in vivo testing of *S. pneumoniae* isolates with genetic mutations in the 23S rRNA and L22 riboprotein gene alleles [2]. The overall effects of telithromycin regimens mimicking human exposures on the *S. pneumoniae* isolates in these in vivo studies were statistically superior to azithromycin. However, one strain varied from the rest in that none of the telithromycin regimens were significantly different compared with azithromycin. To better characterize the activity of telithromycin on these *S. pneumoniae* strains with ribosomal mutations, we undertook this study utilizing in vitro time–kill methodology to assess better the antibacterial activity of this new ketolide.

2. Materials and methods

Telithromycin Batch # 01 N 0111 B, supplied by Sanofi-Aventis Pharmaceuticals (Bridgewater, NJ) was used

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Table 1
Genotypic profile and susceptibility of *Streptococcus pneumoniae* isolates as reported by Farrell et al. [1]

Isolate	Genotype	MIC (μg/mL)	
		Telithromycin	Azithromycin
P1080014	L22 G95D and A2059G	0.06	>64
P1660008	A2058G	0.25	>64
P1025028	A2059G	0.03	>64
P1008006	C2611G	0.015	8

MIC, minimum inhibitory concentration.

throughout the study. Telithromycin was supplied as a white powder and was dissolved in 10% of 95% ethyl alcohol and 0.1 M phosphate buffer (pH 6). Four clinical isolates of *S. pneumoniae* were utilized. These isolates were chosen from the 16 isolates in the PROTEKT study previously noted based on apparent diversity in their genotypic and phenotypic profiles (Table 1). All isolates were kept frozen in skim milk at −80 °C prior to the experiments.

Time–kill analyses were performed on four isolates (P1008006, P1025028, P1080014 and P1660008). Each *S. pneumoniae* isolate was subcultured twice on blood agar plates and a bacterial suspension of 10^8 colony-forming units (CFU)/mL was prepared utilizing a 1.0 McFarland turbidity standard. Mueller–Hinton broth with 5% lysed horse blood was inoculated with the bacterial suspension to a final suspension of ca. 5×10^5 CFU/mL. Telithromycin was added to achieve telithromycin concentrations that were 0.5, 1, 2, 4 and 8 × MIC. Control experiments without active compound were conducted simultaneously with the time–kill studies. Final volumes for each bacterium–drug concentration were 10 mL and these were kept in a shaking water-bath at 35 °C. Samples were taken from each of the bacteria–drug concentrations at 0, 2, 4, 6, 8, 12 and 24 h from the time of adding the drug. Multiple 1:10 dilutions were made in saline, which were then subcultured onto blood agar plates and incubated with 5% CO_2 for 18–24 h. Studies were repeated two to three times on different days and time–kill data are presented as mean ± standard deviation. The limit of detection in this model was 10 CFU/mL.

3. Results

Time–kill data for the four isolates are displayed in Fig. 1. All four isolates had distinct in vitro time–kill activity profiles. However, for all isolates telithromycin at 0.5 × MIC resulted minimal kill, with re-growth occurring by 24 h. Telithromycin at a concentration of 1 × MIC maintained a 1–1.5 log kill with P1025028 and P1080014, whereas re-growth at 24 h was noted for P1660008 and P1008006. For every isolate, except P1008006, telithromycin concentrations of 2, 4 and 8 × MIC decreased the bacterial count over time, and this was concentration-dependent. For the first several hours, telithromycin at 2 and 4 × MIC of P1008006 inhibited growth, but the isolate increased by 2 logs in bacterial density to the level of the control; the 8 × MIC exhibited less than a $1\frac{1}{2}$ log kill.

Over the range of concentrations tested, telithromycin had a concentration-dependent effect against all isolates, although to different degrees. The extent of telithromycin bacterial kill did not reach the level of bactericidal activity, with the exception of activity against one isolate, P1080014. Only against this double-hit mutant was telithromycin bactericidal at all concentrations above the MIC, including 2, 4 and 8 × MIC. Overall, concentrations that were 2, 4 and 8 × MIC resulted in a 2–3 log kill at 24 h with all isolates except P1008006. For this isolate, bacterial kill was seen only at 8 × MIC, which resulted in a maximum of 1 log kill over the 24-h study period.

4. Discussion

Previously conducted time–kill studies with telithromycin have demonstrated the agent's bactericidal activity against *S. pneumoniae* with a variety of phenotypic profiles, including resistance both to β-lactams and macrolides [3,4]. Moreover, these studies demonstrated that this activity was irrespective of the genotypic resistance profile (e.g. *mef*- or *erm*-mediated). At present, macrolide-resistant *S. pneumoniae* isolates testing negative for methylase and efflux mechanisms appears to be exceedingly rare (<2%) [1,5]; however, despite these new mechanisms of resistance, telithromycin appears to display potent microbiological activity [5].

To assess the kill profile of telithromycin against pneumococci harbouring these novel mechanisms of macrolide resistance, time–kill studies were conducted. These first data investigating this new resistance profile reveal that telithromycin exposures as studied demonstrate concentration-dependent killing, although bacterial killing was detected to varying degrees. Against one strain, P1080014, telithromycin was bactericidal at all concentrations above the MIC. For isolate P1008006, whilst telithromycin decreased bacterial density over time with higher multiples of the MIC, only the 8 × MIC exposure produced more than 1 log CFU/mL kill.

Of the four isolates tested, strain P1008006 possessed a mutation at a different location in the gene encoding the 23S rRNA than the other three isolates [1]. As noted above, a diminished kill even at 8 × MIC was noted for this organism. Conversely, in our recent study that simulated in vivo exposures in man, telithromycin successfully reduced the bacterial density of this isolate to a degree similar to that of the remaining isolates [2].

The greatest overall kill was observed for isolate P1080014 both under in vitro and in vivo conditions. In vitro, both P1660008 and P1025028 were slow growing, requiring 8–12 h to reach a maximum density of 10^8 CFU/mL. In vivo, the growth of P1660008 was not consistent with the other isolates, whereas P1025028 appeared to replicate adequately