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Assessment of the efficacy of telithromycin simulating human exposures against *S. pneumoniae* with ribosomal mutations in a murine pneumonia model

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Abstract

Telithromycin (TEL) is a ketolide antimicrobial agent with in vitro activity against *Streptococcus pneumoniae* (SPN), including macrolide resistant strains. The purpose of this study was to assess the efficacy of TEL against clinical SPN isolates with various genotypic mutations including the newly recognized ribosomal mutations. Pneumonia was induced in either immunocompetent and immunosuppressed mice. Six isolates were included in the study and all were resistant to azithromycin (AZI) by MIC testing. Three oral regimens of TEL were chosen to simulate the human pharmacokinetic (PK) exposures observed in young healthy, healthy elderly (≥65 years), and infected subjects. An additional group was given AZI in human simulated doses. Bacterial density in lung was determined after each treatment. Telithromycin administered simulating infected patients showed greater efficacy (i.e., change in log CFU) than the azithromycin treated group for all isolates except P1660008. The immune system was responsible for increased efficacy (ranging from 45–146%) for all but one of the telithromycin treatment regimens. Unlike other isolates studied in this in vivo model, P1660008 displayed a highly variable response to therapy, such that the reductions in CFU were not consistent with the microbiological and PK profiles of either compound. For all other isolates, the activity of AZI was comparable with untreated controls. Human simulated exposures of TEL displayed 0.5–3.4 log kill in vivo despite the ribosomal mutations studied. These data support the in vivo efficacy of TEL against a variety of genotypic resistance profiles observed in pneumococci, however, additional studies are required to fully characterize the killing profile of the compound against these recently determined ribosomal mutations.

Keywords: Telithromycin; *S. pneumoniae*; Antimicrobial resistance; Ribosomal mutation; Experimental infection model

1. Introduction

Over the last decade increasing resistance has been observed in *Streptococcus pneumoniae* (SPN) to many of the frequently used treatment options. Rates of resistance to penicillin have been steadily rising since the late 1980s [1]. Also on a steady rise is the resistance to macrolides by these pathogens. SPN resistance to macrolides occurs primarily due to one of two mechanisms of resistance. The *mef* mediated resistance, where there is active efflux of the macrolide or the *erm* mediated resistance, where there is modification of the drug-binding site. This modification primarily involves dimethylation, which occurs at the 2058 position on the 23S rRNA.

Prospective Resistant Organism Tracking and Epidemiology for the Ketolide Telithromycin (PROTEKT) is a global, multicentre surveillance study of respiratory tract pathogens that collects isolates longitudinally. In evaluating macrolide-resistant SPN isolates from the PROTEKT 1999–2000 study, it was found that 1.5% (16/1043) repeatedly tested negative

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for the common efflux and methylase genes associated with macrolide resistance. These isolates had target modifications in domains V of 23S rRNA and in the genes encoding riboprotein L22 [2].

The objective of this study was to characterize the in vivo activity (i.e., change in log CFU) of telithromycin (TEL) against SPN with ribosomal mutations by simulating human exposures in a murine pneumonia model in both immunocompetent and immunosuppressed hosts.

2. Materials and methods

2.1. Antimicrobial test agent

Telithromycin, supplied by Aventis, (lot #01 N 0111 B) was used throughout the study. TEL was supplied as a white powder and was dissolved in 10% of 95% ethyl alcohol and 0.1 M phosphate buffer (pH = 6), which was administered orally via gastric lavage for in vivo studies. Commercially available azithromcin (AZI) (Zithromax®) oral powder for suspension was reconstituted with sterile water as per package insert and administered orally via gastric lavage to mice.

2.2. Bacterial isolates and susceptibilities

Six clinical isolates of SPN were utilized throughout the study; Table 1 lists their phenotypic and genotypic profiles. Four isolates with the following genotypes were chosen from the PROTEKT study P1080014—L22 G95D and A2059G; P1660008—A2058G; P1025028—A2059G; P1008006—C2611G. An additional two clinical isolates with the common efflux and methylase genes were utilized, isolate #95 with dimethylation of 2058 (e*rm*B), and isolate #102 with an active efflux mediated resistance (*mef*A). All isolates were stored in skim milk at −80 °C.

2.3. Lung infection model

Female specific-pathogen free ICR mice weighing approximately 25 g, supplied by Harlan Sprague Dawley Inc. (Indianapolis, IN), and CBA/J mice, which weighed approximately 20 g, supplied by National Cancer Institute/Charles River (Maryland), were used in this study. The mice were allowed to acclimate for seven days prior to handling and allowed food and water ad libitum. The Hartford Hospital (Hartford, CT) Institutional Animal Care and Use Committee approved the methodology for use of the animals.

ICR mice were rendered neutropenic (i.e., immunosuppressed) by two 0.2 ml intraperitoneal (i.p.) injections of cyclophosphamide 150 mg/kg (Cytoxan®, Bristol-Myers Squibb) on days four and one prior to the inoculation of the mice. In order to compare with an infection established in the presence of functional white blood cells CBA/J mice were utilized [3]. This strain of mice has been shown to succumb to pneumococcal infection in the presence of an immune system and thus does not require cyclophosphamide induced neutropenia to establish infection. However, when neutropenia is induced with this chemotherapeutic agent in the CBA/J strain, the bacterial growth and antimicrobial outcomes are similar to those observed in the neutropenic ICR mice [4].

Infecting organisms were subcultured twice on blood agar plates and incubated each time with CO_2 for 18–24 h prior to use. A bacterial suspension equal to a 3.0 McFarland turbidity standard ($\sim 3 \times 10^8$ CFU/mL) was prepared from the overnight growth plates. Mice were anaesthetized using 2–4% (v/v) isoflurane and oxygen to a point in which their respirations were equal to or greater than one breath per second. At this point, a mouse was held in a vertical position and 0.05 mL of the inoculum was placed into the mouth, immediately covering the nostrils to ensure a proper gasp of air through the mouth for aspiration of the inoculum into the lungs. The infected mice were then placed in an O_2 enriched chamber to recover and randomly placed in either control or treatment groups.

Dosing was initiated in mice 12–14 h after inoculation. Mice were divided into one of six groups (6–8 mice per group): three TEL treatment groups, one AZI and two control groups. The first control group was sacrificed at the initiation of dosing, in order to determine the bacterial density prior to the initiation of therapy. The remaining groups were dosed with TEL, AZI or blank diluent (10% of 95% ethyl alcohol with 0.1 M phosphate buffer 9, pH = 6) for 24 h and then sacrificed. Mice were sacrificed via CO_2 inhalation followed by cervical dislocation. The lungs were harvested using an aseptic technique. The five lung lobes were carefully extracted taking care not to remove any bronchi and collectively homogenized with 1 mL of 0.9% saline. The homogenate was serially diluted, then streaked onto growth media in spirals using the AutoPlate 4000 (Spiral Biotech, Norwood, MA) and the bacterial density for each lung was determined; see Fig. 1 for study schematic. The limit of detection in this model is a bacterial density of 50 CFU/lung.

2.4. Dose selection

Telithromycin pharmacokinetic studies were previously completed in our laboratory in the same species. Since previously conducted pharmacodynamic studies in both mice and

Table 1
Susceptibility data for *S. pneumoniae* isolates

Isolate #	Genotype	MIC (mg/L)	
		TEL	AZI
P1080014	L22 G95D and A2059G	0.06[a]	>64[a]
P1660008	A2058G	0.25[a]	>64[a]
P1025028	A2059G	0.03[a]	>64[a]
P1008006	C2611G	0.015[a]	8[a]
95	*erm*B	0.03[b]	>128[b]
102	*mef*A	0.25[b]	8[b]

[a] Reference [2].
[b] Reference [5].