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In Vitro Bactericidal Activity of Lefamulin Against *Streptococcus pneumoniae* Isolates

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INTRODUCTION & PURPOSE

- Lefamulin, the first pleuromutilin antibiotic for IV or oral use in humans, recently completed phase 3 trials for the treatment of community-acquired bacterial pneumonia (CABP). In the initial study, lefamulin demonstrated non-inferiority to moxifloxacin ± linezolid and showed favorable tolerability and safety profiles
- In a non-inferiority phase 2 study, lefamulin also showed similar efficacy to vancomycin for the treatment of acute bacterial skin and skin structure infections (ABSSSI)[1]
- Like all pleuromutilin antibiotics, lefamulin specifically inhibits prokaryotic protein synthesis by binding to the A- and P- site in the peptidyl transferase center by way of 4 hydrogen-bonds and other interactions that result in tight binding via an induced fit mechanism[2]
- Lefamulin is highly active *in vitro* against pathogens that commonly cause CABP including *Streptococcus pneumoniae, Haemophilus influenzae*, *Moraxella catarrhalis, Staphylococcus aureus*, *Mycoplasma pneumoniae, Chlamydophila pneumoniae* and *Legionella pneumophila*, and its activity is not influenced by resistance to other antimicrobial classes[3-6]
  - The lefamulin MIC_{90} (0.002 µg/mL) for macrolide-resistant *M. pneumoniae* strains was the lowest among all drugs tested. Minimum bactericidal concentrations were within 2 dilutions of the MIC values, indicating lefamulin's bactericidal effect[6]
- There are a number of factors that influence a given antibiotic's clinical effectiveness, such as the site or type of infection, underlying disease, an antibiotic's pharmacokinetic and pharmacodynamics properties, and the mechanism of action of the antibiotic.[7] For some infections, such as serious bloodstream infections, bactericidal antibiotics may be associated with improved outcomes; whereas in other types of infections, the categorization of bacteriostatic vs bactericidal is likely less clinically relevant[8]
- The objective of this analysis was to determine the bactericidal activity of lefamulin against *S. pneumoniae*

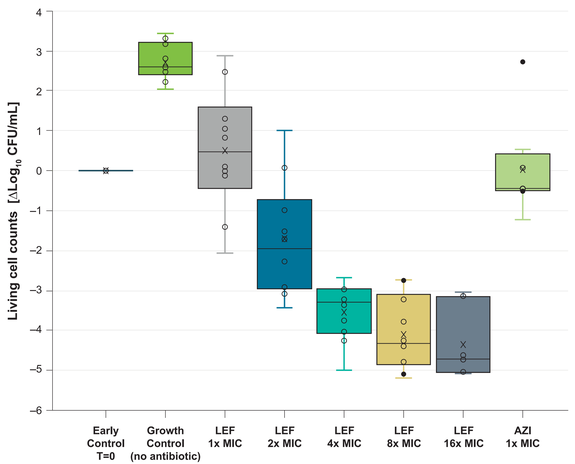
METHODS

- Kill-curves were determined at lefamulin concentrations 1- to 16-fold the MIC and measured by broth microdilution according to Clinical and Laboratory Standards Institute guidelines
  - A *S. pneumoniae* colony was inoculated into 10–20 mL cation-adjusted Mueller Hinton Broth (CAMHB) supplemented with 5% lysed horse blood to a starting turbidity of 0.5 McFarland standard (~10^6 colony forming units [CFU]/mL)
  - Cultures were incubated with shaking at 37°C for 8 hours and living cell counts were determined as CFUs/mL
- Azithromycin, a macrolide with bacteriostatic activity, was included as a control at 1-fold its MIC. The growth control did not include antibiotics
- The maximum duration of incubation was 8 hours due to autolysis observed for the growth controls at 24 hours
- 10 *S. pneumoniae* strains were assayed: ATCC49619, ATCC6303, ATCC10813, and 7 clinical isolates collected in 2010 showing lefamulin MICs of 0.015–0.12 mg/L **(Table 1)**

RESULTS

- Lefamulin displayed bactericidal activity against all strains tested with dependency on concentration and time of incubation
  - Living cell counts of all strains were reduced by ≥3 log units within 8 hours of incubation at 8- to 16-fold lefamulin MIC corresponding to 0.12–1.92 mg/L **(Figure 1)**
  - At 4-fold MIC, lefamulin was bactericidal against 9 of 10 strains with a mean kill rate of –3.35 log CFU/mL
- The kill curves for *S. pneumoniae* clinical strain B1378, and reference strain ATCC6303 demonstrated that lefamulin concentrations ≥1-fold the MIC (≥0.06 µg/mL) were bactericidal, resulting in ≥5-fold log reduction in living cell counts **(Figure 2)**
- The average concentration required for *in vitro* bactericidal activity at 8 hours was 0.5 µg/mL lefamulin
  - Concentrations reported in previous pharmacokinetic analyses in human plasma and epithelial lining fluid following IV administration of lefamulin have well exceeded this value[9]

Figure 1. Concentration Response of Lefamulin Against *S. pneumoniae* at 8 Hours



AZI=azithromycin; CFU=colony forming units; LEF=lefamulin; MIC=minimum inhibitory concentration.

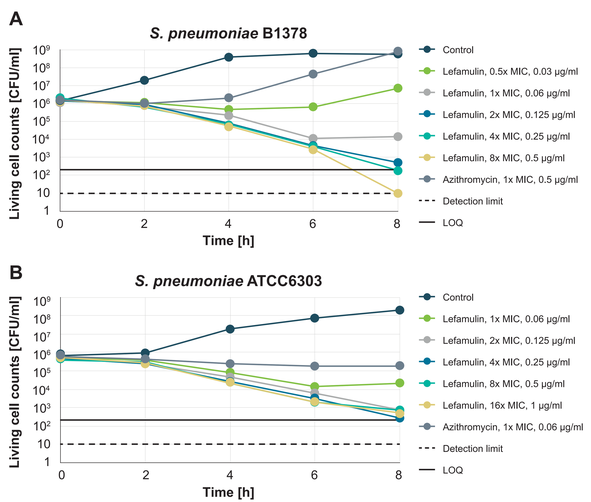
RESULTS (continued)

Table 1. Lefamulin and Azithromycin MICs Against *S. pneumoniae* Strains

Strain	Source	MIC (µg/mL)	
		Lefamulin	Azithromycin
B1379	Clinical isolate, SENTRY 2010	0.015	2
ATCC49619	Reference strain	0.03	0.06
B1383	Clinical isolate, SENTRY 2010	0.03	>256[a]
ATCC6303	Reference strain	0.06	0.06
B1378	Clinical isolate, SENTRY 2010	0.06	0.5
B1382	Clinical isolate, SENTRY 2010	0.06	0.06
B1385	Clinical isolate, SENTRY 2010	0.06	0.03
B1386	Clinical isolate, SENTRY 2010	0.06	4
ATCC10813	Reference strain	0.06	0.06
B1389	Clinical isolate, SENTRY 2010	0.12	>256[a]

CFU=colony forming units; MIC=minimum inhibitory concentration; SENTRY=SENTRY Antimicrobial Surveillance Program.
[a]Isolates are resistant to azithromycin and were therefore not tested.

Figure 2. Kill Curves for Representative *S. pneumoniae* Strains



CFU=colony forming units; control=growth with no antibiotics; LOQ=limit of quantitation; MIC=minimum inhibitory concentration.

CONCLUSIONS

- Lefamulin demonstrated bactericidal activity against all 10 *S. pneumoniae* isolates tested as was observed for *Mycoplasma pneumoniae* in an earlier *in vitro* study[6]
- Killing was dependent on the lefamulin concentration and time of incubation
- These results correlate with *in vivo* data supporting AUC:MIC as the primary pharmacokinetic/ pharmacodynamic index and support the development of lefamulin for the treatment of CABP

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Acknowledgments and Disclosures

The authors thank JMI Laboratories for provision of the clinical isolates collected during the SENTRY 2010 surveillance. Funding for development of this poster was provided by Nabriva to C4 MedSolutions, LLC (Yardley, PA), a CHC Group company. Susanne Paukner, Gisela Gassner and Astrid Gruss are employees of Nabriva.

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28th European Congress of Clinical Microbiology and Infectious Diseases; April 21–24, 2018; Madrid, Spain