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Efficacy of Lefamulin Against Staphylococcus aureus-Induced Bacteremia in a Neutropenic and Immunocompetent Murine Model

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ABSTRACT

Background: *S. aureus* (SA) is a major, human pathogen that causes invasive, clinical infections, including bacteremia. Lefamulin (LEF) is the first semi-synthetic, pleuromutilin antibiotic for IV and oral use in humans. LEF is currently in Phase 3 trials for the treatment of community-acquired bacterial pneumonia (CABP). LEF specifically inhibits bacterial protein synthesis by binding to the peptidyl transferase center (PTC) via four H-bonds and other interactions at the A- and P-site resulting in an "induced fit." LEF has been shown to be highly-active against bacterial pathogens causing bacteremia, including SA. This study investigated the efficacy of LEF and comparators against SA in a neutropenic and immunocompetent murine bacteremia model.

Methods: Experimentally-induced MSSA bacteremia (inoculum ~2x10^7 CFU/mouse) was established in immunocompromised and immunocompetent mice. Infected mice received a single, subcutaneous dose of either LEF or comparator (Table 1) 1 h post-inoculation, mimicking human therapeutic exposures. A control group of infected mice were sacrificed directly before treatment to establish a baseline CFU count and comparison with the bacterial load of treated animals 24 h post drug administration.

Results: Irrespective of the immune status, LEF showed superior efficacy to linezolid (LZD) and tigecycline (TGC) against MSSA, reducing the bacterial burden more than 4 log^{10} CFU/mL within 24 h (Table 1). A comparable reduction of bacterial burden was observed between LEF and daptomycin (DAP) or vancomycin (VAN) treatment.

Conclusion: LEF showed comparable therapeutic outcome to DAP or VAN in this acute experimental infection model, while showing superior killing as compared to LZD or TGC. The efficacy of LEF was maintained under neutropenic conditions with >4log_{10} ΔCFU/ml at clinically relevant exposures. This study supports continued evaluation of LEF for as a potential treatment of staphylococcal bacteremia.

INTRODUCTION

Lefamulin is the first representative of pleuromutilin class in clinical development for systemic administration in humans. Pleuromutilins inhibit bacterial protein synthesis of Gram-positive and Gram-negative organisms, as well as atypical respiratory pathogens. Lefamulin effectively and selectively inhibits bacterial translation by binding to the peptidyl transferase center (PTC) via four H-bonds and other interactions at the A- and P-site resulting in an "induced fit." Phase 1 and 2 trials have demonstrated that IV and oral administration of lefamulin are well tolerated. Furthermore, lefamulin (100mg or 150 mg IV q12 hours) showed similar efficacy to IV vancomycin in a clinical, Phase 2 trial in patients with acute bacterial skin and skin structure infections (ABSSI). Currently lefamulin is in late stage development for the treatment of community-acquired bacterial pneumonia (CABP).

METHODS

MSSA (grown at 37° C in MHB for 16 h) suspension in saline was used for intraperitoneal infection of NMRI mice (*n*=8).

The challenging dose was approximately 2 x 10^7 CFU per mouse, which represented a 100 % lethal concentration for systemic infections within 24 h. Mice randomized for the immune deficient infection model were given cyclophosphamide (Endoxan, Baxter, Germany) intraperitoneally twice prior to bacterial challenge. The first dose of 150 mg/kg was given four days before the challenge and the second dose of 100 mg/kg was given one day before the challenge. This pre-treatment regimen resulted in a reliable, transient leukopenia and neutropenia in mice that lasted for three days after the last dose of cyclophosphamide was given.

The antibacterial subcutaneous (SC) challenge was initiated one hour after infection as a single dose. The murine doses of lefamulin (70 mg/kg), daptomycin (22.5 mg/kg), vancomycin (160 mg/kg), linezolid (80 mg/kg) and tigecycline (6.5 mg/kg) were selected to mimic respective therapeutic human exposures.

Prior to treatment (Early Control) and at 24 h after start of therapy designated groups of animals were euthanized for blood titer determination. None of the untreated control animals survived beyond 24 h p.a. Dead animals were included into the analysis with a log_{10} CFU/ml of 7.3. The lower limit of quantification was 1.3 log_{10} CFU/ml. For statistics all values below LLOQ (1.3 log_{10} CFU/ml) were handled as LLOQ/2.

A one way ANOVA was used for statistical analysis (SigmaStat, 3.11). The efficacy of lefamulin compared to the reference compounds was analyzed by Bonferroni´s multiple-comparison procedure. P < 0.05 was considered as statistically significant.

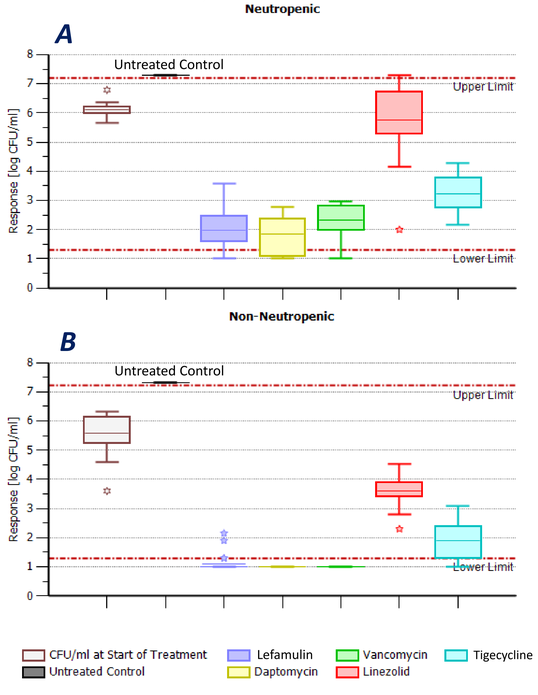
Data were depicted as column plots and box plots using the software package of Phoenix Winnonlin 6.1.

RESULTS

- In the non-neutropenic murine model the efficacy of all tested antibiotics showed a statistically significant decrease of CFU/mL blood compared to the initial bacterial burden in the blood (Early Control; EC) (Figure 1B).
- In the immune deficient animal model only linezolid showed no significant difference in CFU/mL compared to the EC titer (Figure 1A).
- Irrespective of the immune status, the reduction in blood titers caused by lefamulin was > 4 log_{10} CFU/ml and significantly greater than those observed for the bacteriostatic drugs linezolid and tigecycline.
- Lefamulin treatment led to a significant decrease in CFU/ml within 24 h very similar to that of the bactericidal drugs daptomycin and vancomycin, both recommended for the treatment of bacteremia caused by *S. aureus* (Table 1).
- Lefamulin showed *in vivo* bactericidal properties comparable to daptomycin, irrespective of the immune status (Figure 1).

RESULTS cont.

Figure 1: Antimicrobial response box-plot comparing therapeutic doses of lefamulin, daptomycin, vancomycin, linezolid and tigecycline against bacteremia caused by MSSA.



The solid line in the box represents the arithmetic mean value

Table 1: Efficacy of lefamulin and reference antibiotics against *S. aureus* (B9; MSSA; ATCC 49951) in the murine bacteremia model

Compound	Dose [mg/kg/day]	MIC [µg/mL]	*n*	Viable Counts [log_{10} CFU/mL blood] Mean ± SD	Δlog_{10} CFU/mL
non-neutropenic					
Early Control (CFU_{10})	-	-	24	5.58 ± 0.67	±0.00
Untreated (t = 24 h)	-	-	24	> 7.3 [†b]	>1.72
Lefamulin	**70**	**0.06**	**32**	**1.08 ± 0.26 [a]**	**-4.50**
Vancomycin	160	1	16	1.00 ± 0.00 [ac]	-4.58
Linezolid	80	2	16	3.61 ± 0.57 [ab]	-1.97
Daptomycin	22.5	0.25	16	1.00 ± 0.00 [ac]	-4.58
Tigecycline	6.5	0.25	16	1.91 ± 0.68 [ab]	-3.67
neutropenic					
Early Control (CFU_{10})	-	-	24	6.12 ± 0.22	±0.00
Untreated (t = 24 h)	-	-	24	> 7.3 [†b]	>1.18
Lefamulin	**70**	**0.06**	**32**	**1.98 ± 0.68 [a]**	**-4.14**
Vancomycin	160	1	16	2.33 ± 0.62 [a]	-3.79
Linezolid	80	2	16	5.75 ± 1.34 [b]	-0.37
Daptomycin	22.5	0.25	16	1.86 ± 0.62 [a]	-4.26
Tigecycline	6.5	0.25	16	3.21 ± 0.63 [ab]	-2.91

[a] *P* < 0.05 compared with Early Control (Dunnett´s method)
[b] *P* < 0.05 compared with lefamulin (Bonferroni t-test)
[c] All values below LLOQ (1.3 log_{10} CFU/ml) were set to LLOQ/2 (1.0 log_{10} CFU/ml).
[†] Untreated controls did not survive beyond 24 h p.a.

CONCLUSIONS

- **Lefamulin showed therapeutic outcome comparable to DAP or VAN in this acute experimental infection model, while showing superior killing as compared to LZD or TGC.**
- **The efficacy of lefamulin was maintained under neutropenic conditions with >4log_{10} ΔCFU/mL at clinically relevant exposures.**
- **This study supports continued evaluation of lefamulin for as a potential treatment of staphylococcal bacteremia.**

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