

Session: EP075 New antibiotics - new approaches

Category: 5a. Mechanisms of action, preclinical data & pharmacology of antibacterial agents

23 April 2017, 14:12 - 14:17
EP0405

Lefamulin selectively inhibits bacterial protein synthesis

Susanne Paukner^{*1}, Astrid Gruss¹, Rosemarie Riedl²

¹*Nabriva Therapeutics Ag; Microbiology*

²*Nabriva Therapeutics Ag; Medicinal Chemistry*

Background: Lefamulin is the first semi-synthetic pleuromutilin antibiotic for IV and oral use in humans. It is currently in Phase 3 trials for the treatment of community-acquired bacterial pneumonia in adults. Pleuromutilins inhibit translation by binding to the peptidyl transferase center (PTC). The pleuromutilin core binds to the A-site, while the C-14 side chain binds in the P-site. Lefamulin interacts with the PTC via four H-bonds and other interactions resulting in an “induced fit” whereby nucleotides in the PTC shift and further tighten the binding pocket around lefamulin. This study investigated the translation inhibition of bacterial vs. eukaryotic translation to evaluate the relative specificity to bacterial vs. mammalian tissue.

Material/methods: Inhibition of protein synthesis was measured by coupled *in vitro* transcription-translation (TT) using *E. coli* or *S. aureus* ribosomal extracts which measure the expression of functional luciferase. Eukaryotic TT inhibition was evaluated with the rabbit TNT SP6 coupled reticulocyte lysate systems assay. Functional luciferase was detected by the Steady-Glo Luciferase Assay System.

Results: Lefamulin was a potent inhibitor of bacterial translation with an IC₅₀ of 0.58 μM and 0.29 μM for *E. coli* and *S. aureus*, respectively. In contrast, lefamulin was ineffective in inhibiting mammalian protein synthesis (IC₅₀ of 952 μM). Positive controls utilized include retapamulin, a topical pleuromutilin, puromycin, a known non-selective prokaryotic and eukaryotic protein synthesis

inhibitor, and cycloheximide. All controls results were consistent with data in the literature. Translation inhibition results correlate well with the potent activity of lefamulin against Gram-positive and fastidious Gram-negative bacterial pathogens.

Conclusions: This study demonstrated the specificity of lefamulin for the bacterial ribosomes hence ruling out any inhibitory effect on the protein synthesis of mammalian cells. These results are consistent with the known structural differences in the PTC of the bacterial vs. eukaryotic ribosome and correlate with lefamulin's good tolerability profile in clinical trials.

Table: IC₅₀ [μM] (CI₉₅) of lefamulin and comparators in *in vitro* bacterial and eukaryotic TT-assay.

Compound	<i>E. coli</i>	<i>S. aureus</i>	Eukaryotic
Lefamulin	0.58 (0.52-0.64)	0.29 (0.26-0.32)	952 (732-1238)
Retapamulin	0.69 (0.64-0.76)	0.35 (0.32-0.39)	850 (562-1287)
Puromycin	0.39 (0.34-0.46)	0.19 (0.16-0.23)	0.31 (0.27-0.36)
Cycloheximide	>100	>100	0.44 (0.29-0.68)