Background: Lefamulin (LEF) is a pleuromutilin antibacterial approved by the United States (US) FDA for the treatment of community-acquired bacterial pneumonia in adults. In addition to its antibacterial activity, LEF has demonstrated anti-inflammatory activity in an LPS induced lung neutrophilia model in mice. We investigated the anti-inflammatory activity of lefamulin in the H1N1 influenza virus mouse model in comparison to oseltamivir (OTV) and azithromycin (AZM).

Methods: Infection was performed in BALB/c mice by intranasal challenge with ~70 PFU influenza virus H1N1 A/PR/8/1934 (Day 0). Treatment with drugs at clinically relevant doses started on Day 1 (LEF 70 mg/kg/day and 110/140 mg/kg/day, subcutaneous (SC), AZM 30 mg/kg/day, intraperitoneal (IP) and OTV 20 mg/kg/day per oral (PO)) to Day 6. On Days 3 and 6 for analysis of infiltrating lung leukocytes (viable CD45+ cells) by flow cytometry (BD LSRFortessa Aria, Becton, Dickinson & Co), cytokines (BioRad Bio-Plex 200, Bio-Rad Lab) and virus titres. At Day 6 lung tissue was assessed for gross pathology and viral titre, and a small lobe was preserved in fixative for histopathology.

Results: In untreated vehicle control animals, the influenza infection progressed as expected with bodyweight loss, increased cell infiltration into the lung and increased levels of TNF-α, IL-6 at day 3 and 6. Treatment with LEF significantly decreased neutrophils, natural killer cells infiltration in the lung by day 6 at both doses tested (Figure 1A). Cytokine levels in the BALF were significantly reduced on day 3 when the viral load peaked. Furthermore, LEF showed positive effects on lung consolidation and reduction across the study. Treatment with lefamulin significantly decreased the total immune cell infiltration in the lung by day 6 at both doses tested, while that of the anti-inflammatory antibiotic azithromycin and the antiviral oseltamivir groups was similarly high as the vehicle control (Figure 1A).

LEF had a positive effect on lung consolidation and survival across the study. Treatment with lefamulin significantly decreased neutrophils, natural killer cell, CD4 and CD8 T-cell infiltration in the lung in both dose tested, while B cells reduction was only significant for LEF regimen 2 (data not shown).

LEF demonstrated anti-inflammatory activity following acute influenza virus H1N1 infection in mice. LEF was able to significantly reduce the lung immunopathological score and viral pneumonia as well as other diseases of the lower respiratory tract.

LEF treatment resulted in a dose-dependent and significant reduction in bronchial degeneration and alveolar inflammation resulting in an overall significant reduction in histopathological score in dosing regimen 3 (Figure 1B).

Azithromycin, in contrast, did not result in significantly different histopathological scores than the vehicle group (Figure 1B).

Both lefamulin doses resulted in significant decreases of the pro-inflammatory cytokine IL-6 and TNF-α levels in BALF in comparison to the vehicle control on day 3, while the viral load peaked (Figure 1D). Overall, lower cytokine levels at both doses, but more notably at the higher dose (regimen 2) successfully suppressed the development of broncho-interstitial pneumonia induced by influenza virus A/Puerto Rico/8 (H1N1) challenge.

CONCLUSIONS

Lefamulin demonstrated anti-inflammatory activity following acute influenza virus H1N1 infection in mice. Lefamulin was able to significantly reduce the lung immunopathology and improve the clinical outcome by a significant inflammatory reduction in bronchial degeneration and alveolar inflammation.

Results from this study were consistent with that observed in the MIP-2 stimulated lung neutrophilia model that demonstrated reduced cytokine concentrations in BALF and reduced infiltration of the lung by inflammatory monocytes and neutrophils.

Further studies are warranted to evaluate the immunomodulatory potential of lefamulin and its relevance in the treatment of bacterial and viral pneumonia as well as other diseases of the lower respiratory tract.

REFERENCES


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Disclosures

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