ATS 2010
Poster #3506

Superior anti-inflammatory effects of a novel macrolide/fluoroketolide, CEM-101 in monocytic cells

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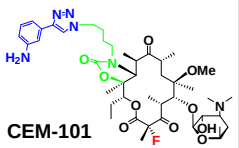
Abstract

Rationale Macrolides are reported to reduce exacerbation of COPD and also show anti-inflammatory effects *in vitro*. However the anti-inflammatory efficacies of current macrolides are not optimum. Here we found that CEM-101, a novel macrolide/fluoroketolide (starting Phase 2) which has activities against wide range of bacteria causing pneumonia, showed more potent anti-inflammatory effects than any other macrolides being marketed. **Methods:** Effects of CEM-101 on PMA-induced MMP9 production and LPS-induced IL-8 and TNFα release in U937 monocytic cells have been evaluated and compared with the effects of erythromycin (EM), clarithromycin (CAM), azithromycin (AZM) and telithromycin (TEL). **Results**: CEM-101 concentration-dependently inhibited MMP9/IL-8/TNFα production in U937 with IC_{50}s of 28.9 ± 1.6 μM, 78.2 ± 9.5 μM and 41.6 ± 1.9 μM respectively. In contrast, CAM had 10 times less anti-inflammatory effects than CEM-101. EM, AZM and TEL did not show significant anti-inflammatory effects.
Conclusion: CEM-101 shows better anti-inflammatory profiles compared with macrolides currently used in clinic, and will be a promising anti-inflammatory and anti-bacterial macrolide/fluoroketolide for the treatment of COPD.

Introduction

• Chronic obstructive pulmonary disease (COPD) is characterized by chronic airway inflammation and is caused by a mixture of small airway obstruction and emphysema. As one of the molecular mechanisms of airway inflammation in COPD, there is increased expression of specific inflammatory genes, such as IL-8, TNFα, and matrix metalloproteinase 9 (MMP9) [1, 2].
• Clarithromycin has been reported to reduce IL-8 and TNFα productions in sputum from COPD patients [3]. Further, erythromycin and clarithromycin therapy reduced exacerbation of COPD [4, 5]. These findings indicate that macrolides have anti-inflammatory properties independently of their anti-bacterial effects and might exert an anti-inflammatory effect in COPD as well as other inflammatory airway disease (e.g. diffuse panbronchiolitis, bronchiectasis).
• A novel macrolide/fluoroketolide, CEM-101 which was developed by Cempra Pharmaceutical, Inc., has more active anti-bacterial effect than other macrolides currently in use [6]. We confirmed whether CEM-101 exerts superior anti-inflammatory effects compared with other macrolides.



Methods

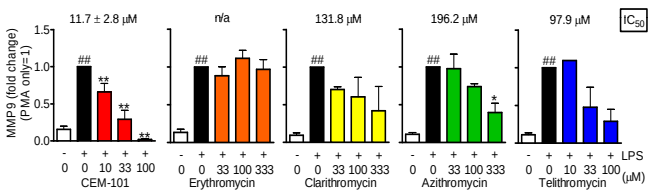
Cells: The human monocytic cell line U937 was treated with CEM-101 or other macrolides (erythromycin, clarithromycin, azithromycin and telithromycin) prior to stimulation with PMA or LPS. U937 cells were differentiated into an adherent macrophage-like morphology by exposure to PMA as needed.
Cytokine ELISA: LPS-induced IL-8 and TNFα concentrations were determined by sandwich ELISA (R&D Systems Europe). IC_{50} values for macrolides were calculated using Prism 4.0 (GraphPad Software Inc.).

Zymography: MMP9 enzyme activity was measured by gelatin zymography. 5 μl of supernatants were diluted with 5 μl Laemli sample buffer (Bio-Rad) and were loaded on a Novex® 10 % Zymogram (Gelatin) gel (Invitrogen). After electrophoresis (90 min, 125 V, 35 mA, 5 W) gels were incubated with 1× Novex® zymogram renaturing buffer (Invitrogen) for 30 min at room temperature with gentle agitation. Gels were then rinsed in 1× Novex® zymogram developing buffer (Invitrogen) for 30 min at room temperature with gentle agitation prior to overnight incubation in the developing buffer at 37 °C. After incubation the gels were stained using the colloidal blue staining kit (Invitrogen) with buffer containing 20 % methanol and 70 % distilled water to visualize the zymogen bands. Relevant band intensities were quantified by densitometric analysis using the UVP GelDoc-It system.

Aim

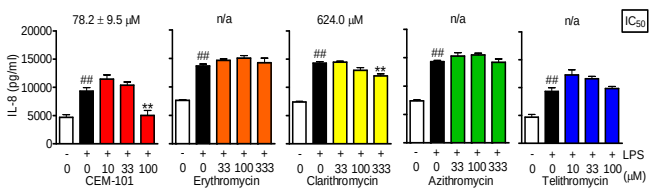
To explore whether a novel macrolide/fluoroketolide, CEM-101, has more potent anti-inflammatory effects than other macrolides currently used clinically.

Results 1: Effects of macrolides on PMA-induced MMP9 production



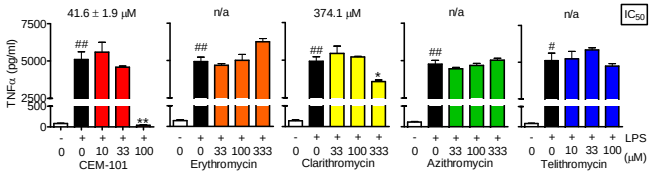
U937 cells pre-treated with macrolide compounds (CEM-101 and Telithromycin (TEL); 10 to 100 μM, Erythromycin (EM), Clarithromycin (CAM), and Azithromycin (AZM); 33 to 333 μM) for 1 hr, followed by PMA (50 ng/ml) treatment for 48 hrs. After 48 hrs supernatants were collected for zymography. MMP9 enzyme activity was measured by gelatin zymography. Data are expressed as fold changes against positive control treated with PMA only. IC_{50}s for each macrolide on PMA-induced MMP9 were calculated using Prism. Values represent means of three experiments ± SEM. $^{\#\#}$ $p < 0.01$ (vs. non-treatment control), * $p < 0.05$, ** $p < 0.01$ (vs. positive control treated with PMA only). n/a; not applicable.

Results 2: Effects of macrolides on LPS-induced IL-8 production



PMA-differentiated U937 cells were pre-treated with macrolide compounds (CEM-101 and Telithromycin (TEL); 10 to 100 μM, Erythromycin (EM), Clarithromycin (CAM), and Azithromycin (AZM); 33 to 333 μM) for 1 hr, followed by LPS (100 ng/ml) stimulation for 4 hrs. LPS-induced IL-8 release was evaluated by ELISA. IC_{50}s for each macrolide on LPS-induced IL-8 production were calculated using Prism. Values represent means of three experiments ± SEM. $^{\#\#}$ $p < 0.01$ (vs. non-treatment control), ** $p < 0.01$ (vs. positive control treated with LPS only). n/a; not applicable.

Results 3: Effects of macrolides on LPS-induced TNFα production



PMA-differentiated U937 cells were pre-treated with macrolide compounds (CEM-101 and Telithromycin (TEL); 10 to 100 μM, Erythromycin (EM), Clarithromycin (CAM), and Azithromycin (AZM); 33 to 333 μM) for 1 hr, followed by LPS (100 ng/ml) stimulation for 4 hrs. LPS-induced TNFα release was evaluated by ELISA. IC_{50}s for each macrolide on LPS-induced TNFα production were calculated using Prism. Values represent means of three experiments ± SEM. $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$ (vs. non-treatment control), * $p < 0.05$, ** $p < 0.01$ (vs. positive control treated with LPS only). n/a; not applicable.

Summary/ Conclusion

• CEM-101 remarkably reduced PMA-induced MMP9 production in U937 cells. The IC_{50} value for CEM-101 on MMP9 production (11.7 ± 2.8 μM) was 10 times superior compared to IC_{50}s for clarithromycin, azithromycin and telithromycin. On the other hand, erythromycin did not decrease MMP9 production even at higher concentration.

• CEM-101 dose-dependently inhibited LPS-induced IL-8 and TNFα production with IC_{50}s of 78.2 ± 9.5 μM, 41.6 ± 1.9 μM respectively. In contrast, the inhibitory effect of clarithromycin was 10 times less than CEM-101. Erythromycin, azithromycin and telithromycin inhibited neither LPS-induced IL-8 nor TNFα.

• Our findings shows that a novel macrolide, CEM-101 exerted superior anti-inflammatory effects than any other macrolides available clinically, which might be due to enhancement of HDAC activity/expression by CEM-101 (ATS 2010, Poster #3527). CEM-101 will be a promising anti-inflammatory drug and a viable option for the treatment of COPD.

References

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Funded by Cempra Pharmaceuticals, Inc., Chapel Hill, NC 27517