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 IC50 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 azithromycin 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). IC50 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 7.5% Tris-HCl gel (Invitrogen). After electrophoresis (90 min, 125 V, 35 mA, 5 W) gels were incubated with 1x Novex® zymogram renaturing buffer (Invitrogen) for 30 min at room temperature with gentle agitation. Gels were then rinsed in 1x 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 cellulose 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 h, followed by PMA (50 ng/ml) stimulation for 4 hrs. MMP9 enzyme activity was measured by gelatin zymography. Data are expressed as fold changes against positive control treated with PMA only. IC50 for each macrolide on PMA-induced MMP9 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 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 h, followed by LPS (100 ng/ml) stimulation for 4 hrs. LPS-induced IL-8 release was evaluated by ELISA. IC50 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

- U937 monocytic 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 h, followed by LPS (100 ng/ml) stimulation for 4 hrs. LPS-induced TNFα release was evaluated by ELISA. IC50 for each macrolide on LPS-induced TNFα production were calculated using Prism. Values represent means of three experiments ± SEM. *p < 0.05, **p = 0.05 (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 IC50 value for CEM-101 on MMP9 production (11.7 ± 2.8 μM) was 10 times superior compared to IC50 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 IC50 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 show 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**


