A Novel Macrolide/fluoroketolide, Solithromycin Exerts Superior Anti-inflammatory Effect via NF-κB Inhibition in COPD Cells

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Abstract

Background: Macrolides are reported to reduce exacerbation of chronic obstructive pulmonary disease (COPD) and also show anti-inflammatory effects *in vitro* and *in vivo*. However the anti-inflammatory efficacies of current macrolides are not optimal. In this study, we evaluated the anti-inflammatory effects of solithromycin (CEM-101), a novel macrolide/fluoroketolide, and those of other macrolides commercially available.

Methods: Effects of solithromycin on LPS-induced TNFα and/or CXCL8 release, PMA-induced MMP9 activity and NF-κB activity under oxidative stress have been evaluated and compared with the effects of erythromycin, clarithromycin and azithromycin in human monocytic U937 cells and PBMCs obtained from COPD patients. TNFα and CXCL8 were measured by ELISA. MMP9 levels were determined by zymography and NF-kB activity was evaluated by NF-kB-DNA binding assay. We also examined effect of solithromycin on airway neutrophilia in mice exposed to cigarette smoke for 12 days.

Results: Solithromycin inhibited LPS-induced TNFα/CXCL8 production and MMP9 activity in U937 cells with IC_{50} values of 78, 42 and 15 μM, respectively, which was more potent than any other macrolide. In addition, solithromycin suppressed TNFα release and MMP9 activity in PBMCs from COPD patients at 10uM, which is 100 times more potent than other macrolides. Activated NF-κB due to oxidative stress (H_2O_2, 200 μM) was completely reversed by solithromycin. Solithromycin (100mg/kg,po) also inhibited cigarette smoke-induced neutrophilia, which is corticosteroid insensitive.

Conclusion: Solithromycin showed better anti-inflammatory profiles compared with macrolides currently used in clinic, and could be a promising anti-inflammatory and anti-microbial macrolide/fluoroketolide for the treatment of COPD.

Introduction

Chronic obstructive pulmonary disease (COPD) is a well-known age-associated disease, which is a major and increasing global health problem with enormous amount of expenditure of indirect/direct health care costs[1]. Chronic obstructive pulmonary disease (COPD) is characterized by largely corticosteroid insensitive progressive small airway inflammation that occurs under oxidative stress. Current therapies are inadequate and no treatments reduce disease progression or mortality. Therefore there is urgent need for new therapies for COPD.

Cigarette smoke is known to be a major cause of COPD, and oxidative stress induces inflammation via NF-kB activation. Oxidative stress down-regulates histone deacetylase 2 (HDAC2) expression via activation of PI3Kδ causing corticosteroid insensitivity in COPD[2]. The main aim was to evaluate anti-inflammatory effects of a novel macrolide/fluoroketolide, solithromycin (CEM-101), and compare the results with macrolides that are currently used.

Materials and Methods

Cells: Human monocytic U937 cells were treated with CEM-101 or other macrolides (erythromicin, clarithromycin and azithromycin) prior to stimulation. U937 cells were differentiated into an adherent macrophage-like morphology by exposure to PMA as needed. PBMCs were isolated from blood obtained from moderate to severe COPD patients.

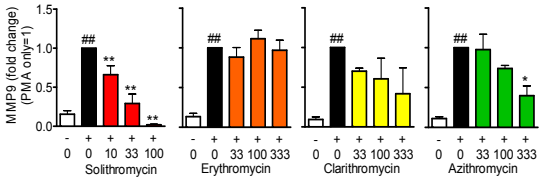
Cytokine ELISA: TNFα-induced IL-8 concentrations were determined by sandwich ELISA (R&D Systems Europe). IC_{50} values for dexamethasone on IL-8 production were calculated using Prism 4.0 (GraphPad Software Inc.) as a marker for steroid sensitivity.

Zymography: MMP9 enzyme activity was measured by gelatin zymography.

NFkB activity: The activation of NF-κB (p65 binding activity to NF-κB binding sequence) was determined using a TransAM NF-κB p65 Assay kit (Active Motif, Inc., Carlsbad, CA), according to the manufacturer's instruction. Results were determined by measuring the spectrophotometric absorbance at 450 nm with a reference wavelength of 655 nm.

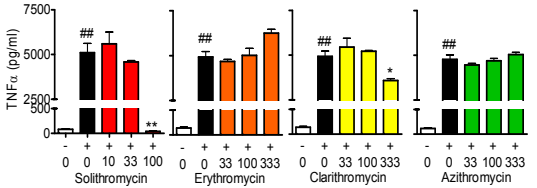
Tobacco smoke mouse model: C57BL/6J mice were exposed to cigarette smoke (4%) for 30 min/day for 12 days. Solithromycin (100mg/kg orally: SOL), dexamethasone (10 mg/kg orally: Dex), a combination of these treatments were administered twice daily for the last 3 days of smoke exposure for 5 animals each. Bronchoalveolar lavage was performed at 24 hours after the last cigarette smoke exposure, and the number of alveolar macrophages and neutrophils were determined from Diff-Quick stained specimens.

Effects on PMA-induced MMP9 production



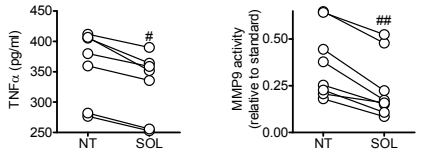
U937 cells pre-treated with macrolide compounds (Solithromycin (SOL); 10 to 100 μM, Erythromycin (EM), Clarithromycin (CAM), and Azithromycin (AZM); 33 to 333 μM) for 1 hr, followed by PMA (50 ng/ml) treatment for 48 hrs. After 48 hrs supernatants were collected for zymography. Data are expressed as fold changes against positive control treated with PMA only. 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).

Effects on PMA-induced TNFα production



PMA-differentiated U937 cells were pre-treated with macrolide compounds (CEM-101; 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. 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.

Effects on IL-8 and MMP9 production in COPD cells



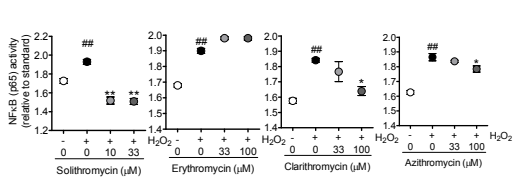
PBMCs obtained from COPD patients were incubated with solithromycin, 10 μM overnight. TNFα release was evaluated by ELISA. MMP9 enzyme activity in supernatants was measured by gelatin zymography. Seven individual values are shown. ## p < 0.01, # p < 0.05 (vs. non-treatment control).

Summary of anti-inflammatory effects

	IC_{50} (μM)			
	Sol	EM	CAM	AZM
IL-8: U937 cell	78	NE@333	507	NE@333
TNFα: U937 cell	42	NE@333	426	NE@333
MMP9 :U937 cell	15	NE@333	118	NE@212
IL-8: COPD cell	P<0.05@10	NE@100	NE@100	NE@100
MMP9: COPD cell	P<0.01@10	NE@100	P<0.01@100	NE@100

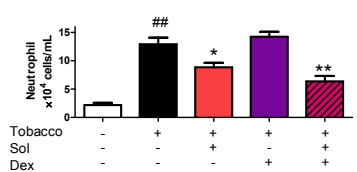
NE: no effect

Effects on H_2O_2-activated NF-kB



PMA-differentiated U937 cells were pretreated with macrolides) for 1 hr, followed by H_2O_2 (200 μM) stimulation for 4 hrs. Binding activity of p65 to NF-κB binding sequence was measured by spectrophotometry. Data was expressed relative to standard (1 mg of recombinant p65). Values represent means of three experiments ± SEM. ## $p < 0.01$ (vs. non-treatment control), * $p < 0.05$, ** $p < 0.01$ (vs. treatment with H_2O_2 only).

Effects on airway neutrophilia in smoke-exposed mice



The number of neutrophils in bronchoalveolar lavage fluid was shown; This neutrophilia was steroid resistant. * $P < 0.05$, ** $P < 0.01$ (vs. smoke control), ## $P < 0.01$ (between air and smoke control).

Conclusions

Solithromycin showed better anti-inflammatory profiles compared with macrolides currently used clinically, and inhibited corticosteroid insensitive neutrophilia in smoke-exposed mice. Therefore, solithromycin could be a promising anti-inflammatory and anti-microbial macrolide/fluoroketolide for the treatment of COPD.

References

1. Vestbo et al., Am J Respir Crit Care Med. 2012 Aug 9.

2: To Y et al., Am J Respir Crit Care Med. 2010 Oct 1;182(7):897-904.