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.

Materials and Methods

Cells: Human monocytes U937 cells were treated with CEM-101 or other macrolides (erythromycin, 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). IC50 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.

NFκB 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.

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. 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-κB activation. Oxidative stress down-regulates the anti-inflammatory genes, which are essential for the maintenance of lung function in COPD. 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

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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.

Effects on PMA-Induced TNFα production

Solithromycin inhibited LPS-induced TNFα (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.

Effects on IL-8 and MMP9 production in COPD cells

Solithromycin inhibited LPS-induced IL-8 (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.

Effects on h2O2-Activated NF-κB

Solithromycin inhibited h2O2-Activated 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.

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

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