A novel macrolide/fluoroketolide, CEM-101, reverses corticosteroid insensitivity under oxidative stress via PI3K pathway inhibition

Y. Kobayashi, C. Rossios, D. Takagi, P. J. Barnes, K. Ito*
Airway Disease Section, NHLI, Imperial College London, UK

Abstract

Rationale We have recently demonstrated that the reduction of histone deacetylase (HDAC) activity via PI3K activation causes corticosteroid (CS) insensitivity in COPD [1-4]. Corticosteroids exert their anti-inflammatory effect by inhibiting the transcription of pro-inflammatory genes through the recruitment of a coactivator complex to the promoter region of the gene. Corticosteroids induce the recruitment of coactivating complexes through the interaction of activated corticosteroid receptor (CSAR) with steroid receptor coactivator (SRC) family members. We have also shown that PI3K signalling also induces the recruitment of coactivating complexes through the interaction of the p85 subunit of PI3K with the SRC family members. In addition, we have previously shown that oxidative stress induces the phosphorylation of Akt, a key downstream effector of PI3K signalling, leading to the activation of CSAR [5]. We have also shown that oxidative stress-induced Akt phosphorylation causes corticosteroid insensitivity in COPD [6]. We have recently demonstrated that oxidative stress-induced Akt phosphorylation causes corticosteroid insensitivity in COPD [1-4].

Methods

• Chronic obstructive pulmonary disease (COPD) is characterized by progressive inflammation in the peripheral lung and oxidative stress. Down-regulation of histone deacetylase 2 (HDAC2) expression and activation of PI3K causes corticosteroid insensitivity in COPD [1-4].

• Akt/PI3K pathway: oxidative stress activates Akt which in turn activates HDAC2 and inhibits inflammation.

• PI3K/Akt pathway: oxidative stress activates Akt which in turn activates HDAC2 and inhibits inflammation.

• HDAC2: HDAC2 is a key enzyme involved in the regulation of gene expression through the removal of acetyl groups from histone tails. HDAC2 is upregulated in COPD and contributes to corticosteroid insensitivity.

• CEM-101: CEM-101 is a novel macrolide/fluoroketolide with dual anti-bacterial and anti-inflammatory properties.

• Aim To explore whether a novel macrolide/fluoroketolide, CEM-101, restores corticosteroid sensitivity compared to other macrolides currently used clinically.

Results

• Results 1: Effects of macrolides on corticosteroid sensitivity under oxidative stress

• U937 cells stimulated with H2O2 (200 μM) overnight were pretreated with macrolides (CEM-101, 10 μM, Erythromycin (EM), Clarithromycin (CA), and Azithromycin (AZM), 100 μM) for 30 min. The cells were treated with dexamethasone (10-10 to 10-4 M) for 45 min, followed by the TNFα stimulation overnight. TNFα-induced IL-8 release was evaluated by ELISA and TNFα values for dexamethasone on IL-8 production were calculated using Prism. Data are expressed as fold changes against non-treatment (NT).

• p-Akt/Akt (% inhibition from reduced level under H2O2): Values represent means of four (A) or three (B) experiments. *p < 0.05, **p < 0.01 (vs. treatment with H2O2 only).

• Results 2: Effects of macrolides on total HDAC activity and HDAC2 mRNA expression under oxidative stress

• A549 cells were pre-treated with macrolides (33 to 330 μM) for 20 min. After H2O2 (200 μM) stimulation for 4 hrs, total HDAC activity was assayed. Data were expressed as % of non-treatment control (NT) (% of control). Data are expressed as fold changes against non-treatment (NT) of CEM-101 (10 μM).

• p-Akt/Akt (% inhibition from reduced level under H2O2): Values represent means of four (A and B) or three (C and D) experiments. *p < 0.05 (vs. NT). Values are expressed as % of non-treatment control (NT) (% of control).

• Results 3: Effects of macrolides on phosphorylation levels of Akt under oxidative stress

• Effects of macrolides on H2O2-induced phosphorylation of Akt in PMA-differentiated U937 cells. Cells were pre-treated with macrolides (33 to 330 μM) for 20 min. After H2O2 (1 μM) stimulation for 30 min, cells were lysed. Data are expressed as fold changes against non-treatment (NT).

• p-Akt/Akt (% inhibition from reduced level under H2O2): Values represent means of four (A) or three (B) experiments.

• *p < 0.05, **p < 0.01 (vs. treatment with H2O2 only).

Summary / Conclusion

• Oxidative stress impaired corticosteroid sensitivity in parallel with reduction of total HDAC activity/HDAC2 mRNA expression and elevation of Akt phosphorylation.

• CEM-101 (10 μM) significantly restored corticosteroid sensitivity in our oxidative stress model. On the other hand, other macrolides did not improve sensitivity even at 10 times higher concentration.

• CEM-101 (10 to 100 μM) reversed total HDAC activity and HDAC2 mRNA expression dose-dependently under oxidative stress. Other macrolides restored them only at higher concentration (333 μM).

• CEM-101 concentration-dependently inhibited H2O2-induced Akt phosphorylation up to 80 %. Although other macrolides inhibited it, the efficacies were less than that of CEM-101.

• This study shows that a novel macrolide, CEM-101 restored corticosteroid sensitivity through PI3K pathway-dependent enhancement of HDAC activity/expression under oxidative stress. CEM-101 might be a viable option for the treatment of COPD or severe asthma, which has less response to corticosteroids.

References


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