

Anti-inflammatory Effects of Macrolides in a Model Relevant to CF Airway Epithelial Inflammation

Abstract

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Macrolide antibiotics prolong survival in patients with diffuse panbronchiolitis, a condition that shares features with cystic fibrosis (CF) lung disease. It has been considered that immunomodulatory properties of macrolides could mediate their therapeutic effects. Previous findings from our laboratory are in accord with this notion. For example, we have reported that exposure of primary cultures of well-differentiated human bronchial epithelia (HBE) to supernatant from mucopurulent material (SMM) from CF airways up-regulates inflammatory and defense response genes. Utilizing a prophylactic protocol, we showed that pretreatment with the macrolide azithromycin (AZT) prevents the SMM-induced up-regulation of genes relevant to airway inflammation, such as MMP9 and MUC5AC (Ribeiro et al, PLoS ONE; 4(6):e5806, 2009). The present study utilized a therapeutic protocol to further the understanding of anti-inflammatory effects of macrolides on expression of inflammatory genes. We tested whether the novel fluoroketolide, Solithromycin (SOLI, CEM-101) modulates the inflammatory responses of HBE exposed to SMM in a similar manner as compared with AZT. In addition, because telithromycin (TELI), a macrolide that is a ketolide, has been shown to inhibit LPS- or *Chlamydomphila pneumoniae*-induced MUC5AC in airway epithelia, we also compared the effects of TELI with those from SOLI and AZT. HBE were mucosally exposed to 30 μ l SMM for 24 hr, followed by serosal exposure to 5, 15 or 30 μ g/ml SOLI, TELI or AZT in presence of SMM. PBS served as a control for SMM-induced effects. SMM-induced matrix metalloproteinase 9 (MMP9) mRNA was inhibited ($p < 0.05$) by all doses of AZT, by 5 and 15 μ g/ml TELI, and slightly inhibited ($p = 0.07$) by 15 μ g/ml SOLI. In contrast, SMM-induced interleukin-8 mRNA was not affected by AZT (in agreement with our published data) or SOLI and only decreased by 20% after 15 μ g/ml TELI. We next evaluated the effect of the macrolides on SMM-up-regulated mucin mRNA levels. AZT and SOLI blunted SMM-induced MUC5AC mRNA in a dose-dependent manner, whereas the effect of TELI was only significantly inhibitory at 30 μ g/ml. On the other hand, all three macrolides exhibited a dose-dependent significant inhibitory effect on SMM-increased MUC5B mRNA levels. As we have previously shown for AZT, preliminary studies with alcian blue-periodic acid Schiff-stained HBE suggest that the inhibitory effect of the macrolides on SMM-induced mucin genes corresponded to decreases in mucin protein expression. Time courses are being currently performed to further evaluate the anti-inflammatory action of the macrolides. Our findings demonstrate that macrolides inhibit the airway epithelial gene expression of MMP9 and mucins in a model relevant to CF. The inhibitory effect on MUC5AC and MUC5B is a novel finding for SOLI. Because CF airways are characterized by chronic inflammation and overproduction of mucins, these effects are likely relevant to the clinical activity of these compounds. Particularly, understanding the mechanisms behind SOLI-dependent gene expression changes should help direct the use of this drug for therapeutic purposes in CF patients.