ANTI-INFLAMMATORY EFFECTS OF MACROLIDES IN A MODEL RELEVANT TO CF AIRWAY EPITHELIAL INFLAMMATION

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Introduction

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 immuno-modulatory properties of macrolides could mediate their therapeutic effects. Previous findings from our laboratory are in accord with this notion. 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 (1). 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 matrix metalloproteinase 9 (MMP9) and MUC5AC (1). The present study utilized a therapeutic protocol to further the understanding of anti-inflammatory effects of macrolides on expression of inflammatory genes in HBE. 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 *Chlamydophila pneumoniae*-induced MUC5AC in airway epithelia (2,3), we also compared the effects of TELI with those from SOLI and AZT.

Effect of Macrolides on IL-8 mRNA and Secreted Protein Levels

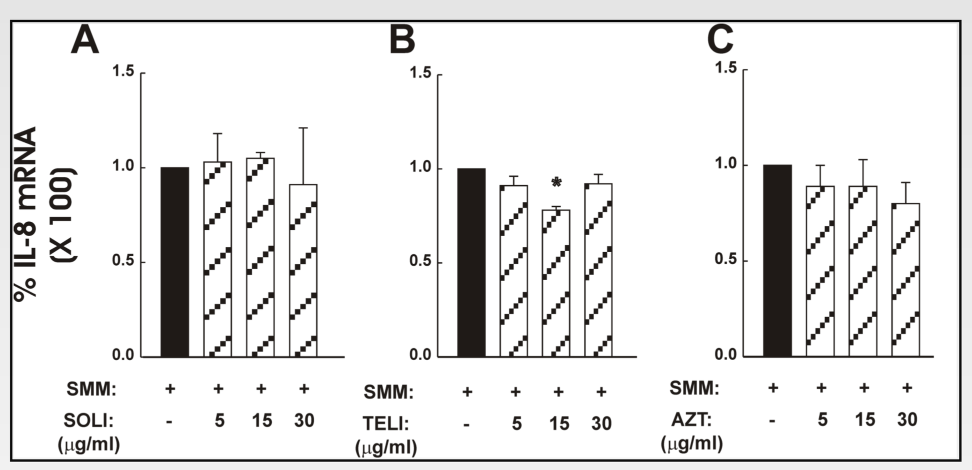

Figure. 1. IL-8 mRNA levels induced by SMM are not affected by SOLI, TELI or AZT. Data are from three individual experiments, utilizing three different human tissue codes, and assayed in duplicate. Data were normalized to the values of SMM-induced gene expression (confirmed by the fold changes of SMM- over PBS-exposed cultures) and represent mean ± SEM. *P<0.05.

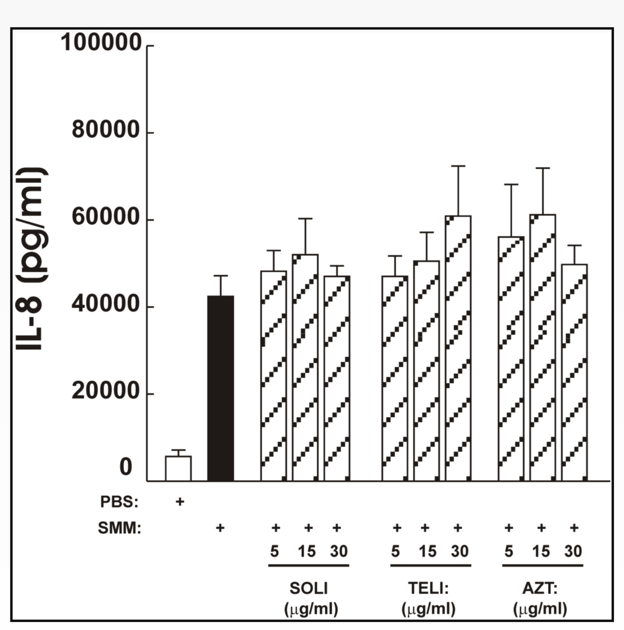

Figure 2. IL-8 secretion induced by SMM is not affected by SOLI, TELI or AZT. Data are from three individual experiments, utilizing three different human tissue codes, and assayed in duplicate. Data represent mean ± SEM.

Methods

HBE were mucosally exposed to 30 µl SMM for 24 hr, followed by serosal exposure to 5, 15 or 30 µg/ml SOLI, TELI or AZT in presence of SMM. PBS served as a control for SMM-induced effects. At the end of treatments, cultures were either fixed with 4% paraformaldehyde (for AB-PAS staining) or their RNA was harvested by standard molecular biology techniques for real time PCR analyses of the following genes: interleukin-8 (IL-8), MMP9, MUC5AC, and MUC5B. A minimum of three individual experiments utilizing 3 different tissue codes corresponding to HBE cultures from three individual subjects were performed.

Effect of Macrolides on MMP9 mRNA Levels

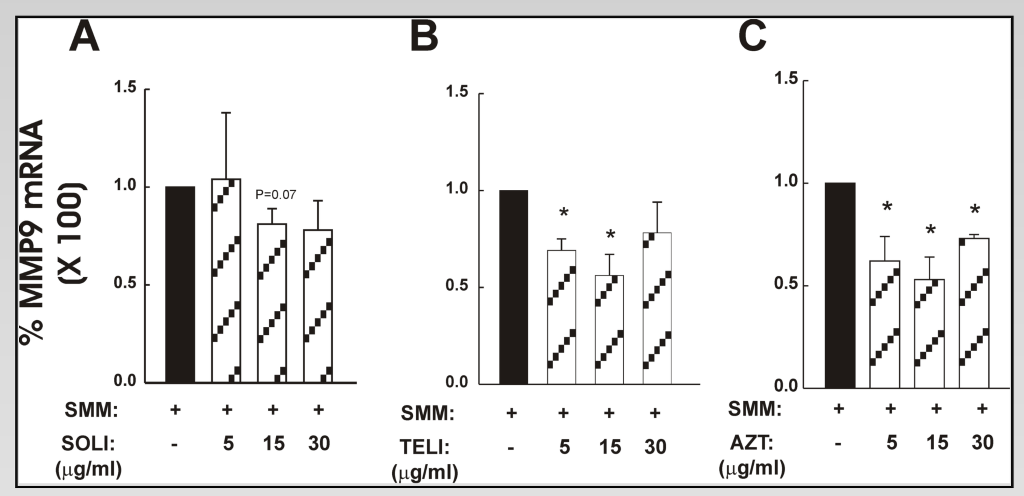

Figure 3. Effect of SOLI, TELI and AZT on SMM-induced MMP9 mRNA levels. Data are from three individual experiments, utilizing three different human tissue codes, and assayed in duplicate. Data were normalized to the values of SMM-induced gene expression (confirmed by the fold changes of SMM-over PBS-exposed cultures) and represent mean ± SEM. * P<0.05.

Macrolides Blunt SMM-Increased Mucin mRNA Levels

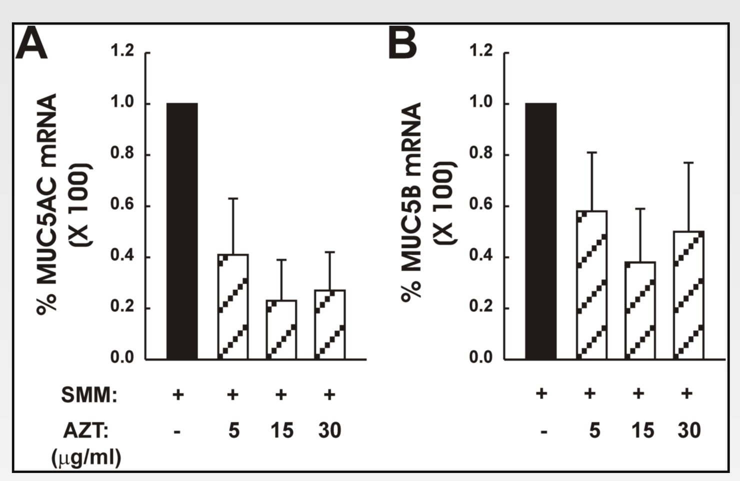

Figure 4. AZT inhibits SMM-increased mucin mRNA levels. Data are from three individual experiments, utilizing three different human tissue codes, and assayed in duplicate. Data represent mean ± SEM. * P<0.05.

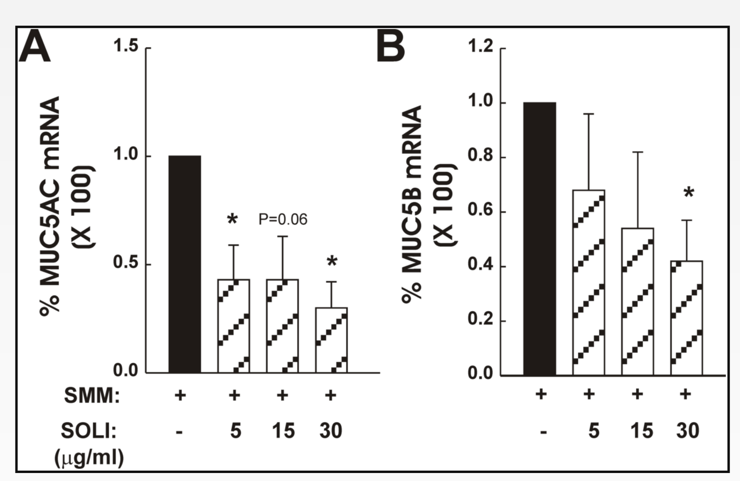

Figure 5. SOLI inhibits SMM-increased mucin mRNA levels. Data are from three individual experiments, utilizing three different human tissue codes, and assayed in duplicate. Data represent mean ± SEM. * P<0.05.

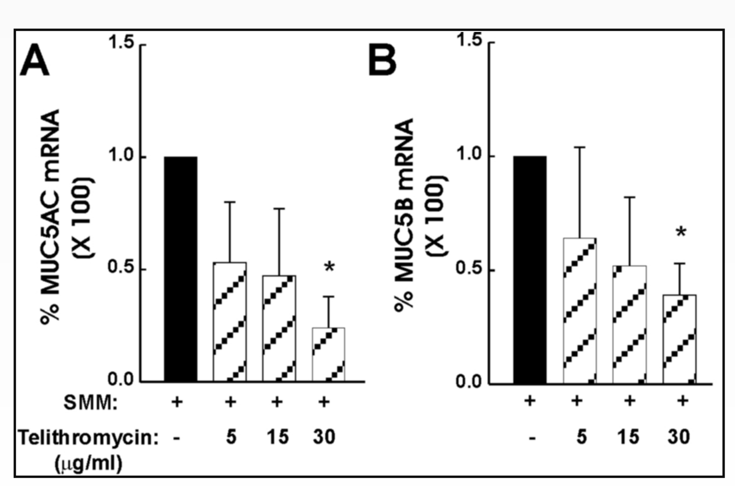

Figure 6. TELI inhibits SMM-increased mucin mRNA levels. Data are from three individual experiments, utilizing three different human tissue codes, and assayed in duplicate. Data represent mean ± SEM. * P<0.05.

Macrolides Inhibit SMM-Increased AB-PAS Staining of HBE

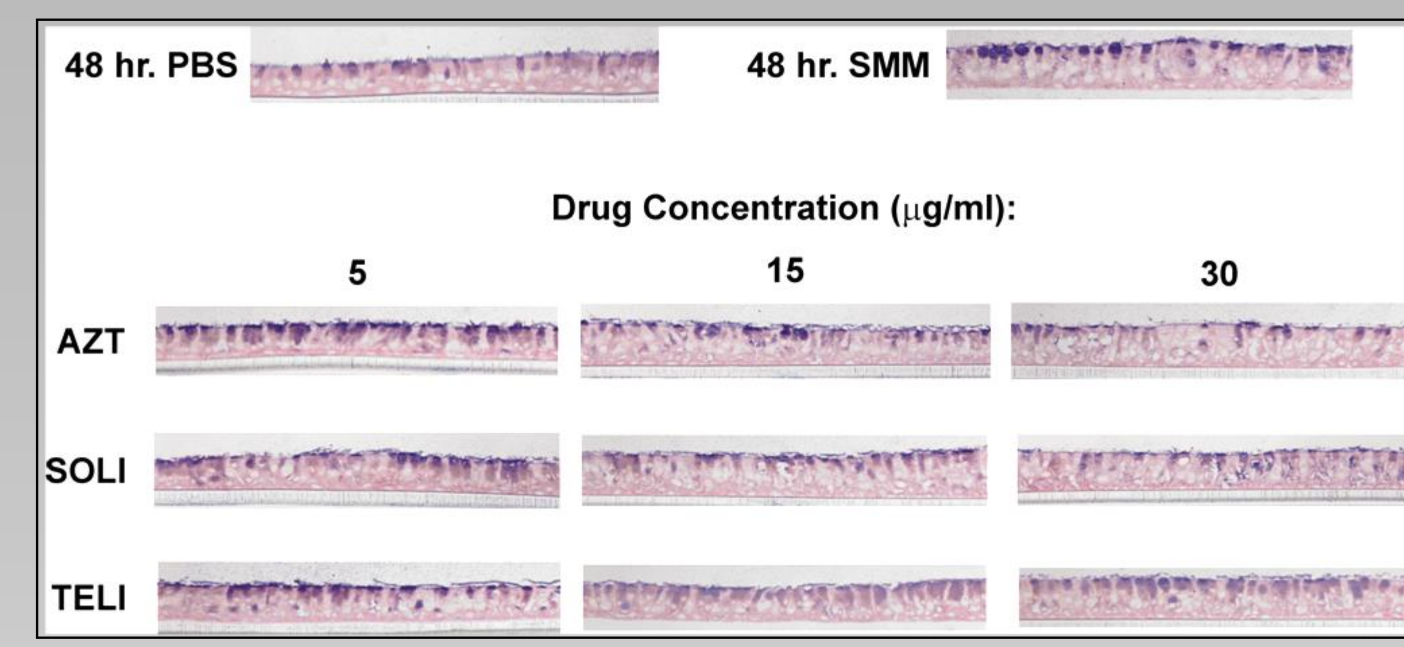

Figure 7. Representative dose response experiment illustrating the inhibitory effect of SOLI, TELI and AZT on SMM-increased alcian blue-periodic acid Schiff **(AB-PAS) stain.**

Summary

SMM-induced IL-8 mRNA was not affected by AZT [in agreement with our published data (1)] or SOLI and only decreased by 20% after 15 µg/ml TELI. In contrast, SMM-induced MMP9 mRNA was inhibited by all doses of AZT, by 5 and 15 µg/ml TELI, and slightly inhibited (p=0.07) by 15 µg/ml SOLI. AZT and SOLI blunted SMM-induced MUC5AC mRNA in a dose-dependent manner, whereas the effect of TELI was only significantly inhibitory at 30 µg/ml. All three macrolides exhibited a dose-dependent significant inhibitory effect on SMM-increased MUC5B mRNA levels. As we have previously shown for AZT (1), studies with AB-PAS-stained HBE suggest that the inhibitory effect of the macrolides on SMM-induced mucin genes correspond to decreases in mucin protein expression. Time courses are being currently performed to further evaluate the anti-inflammatory action of the macrolides.

Conclusion

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. *Funded by Cempra Pharmaceuticals.*

References

1) Ribeiro, C. M. P., Hurd, H., Wu, Y., Martino, M. E. B., Jones, L., Brighton, B., Boucher, R. C., and O'Neal, W. K. (2009): Azithromycin Treatment Alters Gene Expression in Inflammatory, Lipid Metabolism, and Cell Cycle Pathways in Well-Differentiated Human Airway Epithelia. PLoS ONE Jun 5; 4(6):e5806.

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