CATHELICIDINS AND SURFACTANT PROTEINS IN CHRONIC RHINOSINUSITIS: A CLINICAL AND EXPERIMENTAL STUDY

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Abstract

Objectives: To study the expression of cathelicidin antimicrobial peptides (CAMP) and surfactant protein D (SP-D) in patients with chronic rhinosinusitis (CRS) and eosinophilic mucus chronic rhinosinusitis (EMCRS) and by a nasal explant *in vitro* model cultured with fungal allergens.

Methods: Nasal biopsies from 59 CRS and EMCRS patients, stratified into Allergic fungal sinusitis (AFS), Nonallergic fungal eosinophilic sinusitis (NAFES), and Nonallergic nonfungal eosinophilic sinusitis (NANFES) were studied by quantitative real-time (q)RT-PCR, Western blot, immunostaining and ELISA. Nasal tissue from CRS and EMCRS patients were cultured with increasing concentrations of fungal allergens in a nasal explant *in vitro* model for 24 hours and CAMP and SP-D mRNA and protein levels in response to the fungi were determined by qRT-PCR and ELISA.

Results: The expression of CAMP mRNA was significantly increased in EMCRS patients compared to CRS patients ($p=0.0004$). By immunohistochemistry, expression of CAMP was localised to nasal epithelial, submucosal glands and inflammatory subepithelial cells. Western blotting demonstrated the presence of CAMP in the study patients. Culturing nasal explants with fungal allergens demonstrated significant upregulation of CAMP mRNA expression in CRS, but not EMCRS patients, by *Aspergillus* (mean 4-fold increase) and *Alternaria* (mean 6-fold increase) extracts with a significant dose-response effect ($p<0.001$). CAMP protein levels in the nasal tissue from CRS patients increased in response to *Alternaria* ($p<0.05$). In contrast, with EMCRS patients the expression of CAMP peptide in nasal tissue increased with *Aspergillus* ($p<0.001$) but decreased with *Alternaria*. Staining for SP-D was detected in the submucosal glands from the nasal biopsies in all patient groups except for AFS. By ELISA, SP-D was undetectable in AFS and decreased in NAFES, NANFES, and CRS compared to controls. CRS patients cultured with *Aspergillus* and *Alternaria* allergens in the *in vitro* nasal explant model induced significant upregulation

of SP-D mRNA ($p<0.0001$). In contrast, NANFES nasal tissue explants cultured with *Aspergillus* allergens induced downregulation of SP-D and only a modest upregulation of SP-D mRNA to *Alternaria* allergens.

Conclusion: This study demonstrates expression of cathelicidin antimicrobial peptides and surfactant proteins in nasal mucosa supporting its potential role in innate defences against inhaled pathogens. There is significant upregulation of CAMP mRNA in the EMCRS group implying an increased inflammatory state. *In vitro,* CAMP is significantly upregulated at the mRNA and protein level in CRS tissue explants to *Aspergillus* and *Alternaria* allergens. However, EMCRS tissue cultured with *Alternaria in vitro* does not demonstrate increased CAMP at the mRNA or protein level. The expression of SP-D in nasal tissue is reported for the first time. SP-D expression in the CRS, but not the EMCRS group, is upregulated *in vitro* by *Aspergillus* and *Alternaria*. The EMCRS group compared to CRS group demonstrate abnormal CAMP and SP-D expression to common fungal allergens. These important findings in understanding the pathogenesis of chronic rhinosinusitis are discussed in this thesis and may provide potential novel therapies for chronic rhinosinusitis in the future.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university of other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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