S-RM-4

Upregulation of Inducible Nitric Oxide Synthase (INOS) Expression in Human Respiratory Mucosa by *Pseudomonas Aeruginosa (PA)* 1-Hydroxyphenazine (HP)

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PA is a versatile bacterium capable of acquiring multi-resistance against antibiotics and production of a wide variety of toxins. *PA* frequently infects the airways of patients with cystic fibrosis (CF) and severe bronchiectasis and this infection leads to significant clinical deterioration. 1-HP is a yellow degradation product of the PA phenazine toxin, pyocyanin, which slows ciliary beat frequency and downregulates iNOS expression in human respiratory mucosa. We have investigated the effect of 1-HP on human nasal respiratory epithelium and its effect on inducible and endothelial nitric oxide synthase (iNOS and eNOS) expression in human respiratory mucosa. Strips of normal human nasal ciliated epithelium, obtained from the inferior turbinate of 10 different subjects, were decontaminated for 30 minutes in FAD medium containing antibiotics. The cell suspensions were divided into 2 equal aliquots and incubated in FAD medium containing either saline (control) or 1-HP overnight at 37°C and 5% CO_2. After formalin fixation, the cells were centrifuged at 3000rpm for 10 minutes and were embedded in 2% agarose gel before the dehydration and infiltration process. 3μm paraffin sections were used for subsequent morphological and iNOS and eNOS immunocytochemistry study. After computer assisted image analysis, it has been shown that iNOS and eNOS mean± SD intensity of the control group and 1-HP group was 103.8± 33.4 and 95.2± 30.9 ($p=0.02$), and 93.6± 32.12 and 106.2± 39.9 ($p=0.19$) respectively. Moreover, epithelial cells in the test group showed significant epithelial sloughing and loss of cilia compared with control. PA 1-HP upregulates iNOS expression and causes severe structural damage in human respiratory mucosa *in vitro*. Our original observation could lead to further understanding of the pathogenesis of PA infection, currently virtually impossible to eradicate, in the lung of severely affected bronchiectatic patients.

S-RM-5

The Effect of Aging on Nasal Mucociliary Clearance, Beat Frequency and Ultrastructure of Respiratory Cilia

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The increased susceptibility of the elderly to lower respiratory tract infections cannot be fully explained. Although mucociliary clearance, which is affected by ciliary beating and ultrastructure, plays a crucial role in the defence of the airways against inhaled microbes, little is known of the effects of aging on these parameters. We have studied the nasal mucociliary clearance (NMCC) time, ciliary beat frequency and ultrastructure of respiratory cilia on a cohort of healthy volunteers (age range 11-90 years). Ciliary beat frequency of ciliated nasal epithelial cells was obtained using an established photometric method, and NMCC time was measured by using saccharine test. There was a correlation between ciliary beat frequency ($r=-0.48$, $p=0.0001$) and NMCC time ($r=0.64$, $p<0.001$) with increasing age. Transmission electron microscopy examination revealed an increase in the percent of subjects displaying a presence of microtubular disarrangement and single central microtubule with aging ($p=0.002$ and 0.005 respectively). Subjects above 40 years of age had significantly slower ciliary beat frequency, higher percent of ciliary cross sections displaying single tubules, and longer nasal mucociliary clearance time than their counterparts ($p<0.05$). Our original findings might help explain the frequent occurrence of respiratory infections in the elderly.