The influence of smoking on pulmonary influenza infection – an ex vivo study

Authors: Michael CW Chan*, Renee WY Chan*, Judith CW Mak*, JS Malik Peiris*, John M Nicholls*

Affiliations: Centre of Influenza Research*, School of Public Health and Department of Medicine* and Department of Pathology®, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pok Fu Lam, HONG KONG

Background:
The respiratory system is the main target that is affected by cigarette smoking and influenza A virus infection. There are many publications on cigarette smoking as a risk factor during seasonal influenza A virus infection in mice and humans but, the effect of cigarette smoking on the pathogenicity of highly pathogenic avian influenza (HPAI) H5N1 virus has not been studied. As cigarette smoking alters the gene expression and induces structural changes in the respiratory tract and eventually the host immunity, the effect of cigarette smoking in relation to H5N1 infection is therefore a relevant topic for further investigation.

Methodology:
We extracted the epithelium and regions of the pulmonary parenchyma from the bronchi and lungs of patients who had lobectomy performed for primary or secondary carcinoma of the lung. 10 samples were from non-smokers and 10 samples were from chronic smokers. Primary cultures of human bronchial and alveolar epithelial cells were isolated and exposed to 48 hours of cigarette extract as well as sham exposure. Two virus isolates of highly pathogenic avian influenza A subtype H5N1 virus from patients with fatal human disease were used, A/Hong Kong/483/1997 (H5N1), a clade 0 virus from Hong Kong in 1997 and A/Vietnam/3046/2004 (H5N1), a clade 1 virus from Vietnam in 2004. Viruses were initially isolated in Madin-Darby canine kidney (MDCK) cells. Virus infectivity was assessed by titration of tissue culture infection dose 50% (TCID₅₀) in MDCK cells. Human influenza virus A/Hong Kong/54/98 (H1N1) and A/Hong Kong/1174/99 (H3N2) were used for comparison. RNA was also extracted from bronchial and alveolar epithelium for glycosylation array.
**Results:**
Infection of influenza H5N1 virus (A/Vietnam/3046/2004) in the cigarette extract treated well-differentiated human bronchial epithelial cells resulted in greater influenza matrix gene copy than that in mock treated cells. However, our preliminary data in the ex vivo cultures of human bronchi and lungs showed that tissues isolated from chronic smokers had a lower degree of H5N1 virus infection in the lungs but had greater seasonal influenza H1N1 and H3N2 virus infection. Glycosylation array of 96 genes demonstrated 14 genes were >4 fold upregulated, and 21 genes were >4 fold downregulated in smokers and ex-smokers compared to non-smokers in bronchial tissues. In particular genes related to mucin glycosylation were modulated in chronic smokers. Cytokine analysis of differentiated human bronchial epithelial cells suggested a differential increase in the pro-inflammatory cytokine, IL-1-beta secretion when infected with H5N1 virus (A/Vietnam/3046/2004). In summary, our results suggest that cigarette smoke treated human respiratory epithelial cells demonstrated a inhibited viral replication of the HPAI H5N1 virus and may trigger a higher expression of host innate immune response when compared to non smoke extract treated respiratory cells in vitro.