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Martin, L., & Reihill, J. (2019). Promotion of a Protease-Antiprotease Imbalance in the Airways Through Chronic Vaping. *American Journal of Respiratory and Critical Care Medicine, 200*(11), 1337-1339. https://doi.org/10.1164/rccm.201908-1605ED

**Published in:**
American Journal of Respiratory and Critical Care Medicine

**Document Version:**
Peer reviewed version

**Queen's University Belfast - Research Portal:**
Link to publication record in Queen's University Belfast Research Portal

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Promotion of a Protease-Antiprotease Imbalance in the Airways Through Chronic Vaping

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There is ongoing controversy in regard to the safety of electronic cigarettes (e-cigarettes) and vaping. Although originally promoted to help facilitate smoking cessation a number of significant concerns have been highlighted, not least the uptake of these devices by previously non-smoking youths and their high transfer to traditional smoking as a result of nicotine addiction (1).

Electronic nicotine delivery systems (ENDS) are aerosol-generating devices which ‘heat not burn’ a solution containing a complex mixture of solvents and flavouring (a number of which have known toxicity) in addition to nicotine; the final composition of which in the aerosol is determined by temperature (2). Of note, the fine particles delivered by e-cigarettes are similar in size and concentration to tobacco smoke and although the composition differs, the pattern of particle deposition in the lungs is similar (3). A number of studies now report e-cigarette exposure to be associated with airway irritation and inflammation as well as mucus hypersecretion and have been linked to an exacerbation of symptoms in those with chronic airways diseases such as cystic fibrosis (CF), asthma and chronic obstructive pulmonary disease (COPD) (4).

Proteases and their inhibitors play pivotal regulatory roles in most physiological processes required for life. They act as ‘nature’s molecular scissors’, processing other biological molecules leading to the synthesis, activation or degradation of functionally important peptides and proteins and play a vital role in tissue remodelling and wound healing. However, when the normally exquisite control of their action is lost, proteases can be key triggers or amplifiers of many important human diseases such as cancer, cardiovascular disease, rheumatoid arthritis, sepsis, and neurological disorders including Alzheimer’s disease and multiple sclerosis with many proteases well-recognised as potential biomarkers of disease and/or therapeutic targets (5-7). In chronic airways diseases such as CF, COPD and bronchiectasis a protease-antiprotease imbalance has long been associated with tissue injury and disease progression.
Aberrant proteolytic activity due to high levels of neutrophil elastase (NE) in particular, is widely associated with episodes of acute exacerbation and pulmonary decline (8-10).

E-cig vapour extract (ECVE) has been shown to stimulate the release of MMP-9 and CXCL8 from isolated neutrophils as well as an increase in NE and MMP-9 activity (11). MMP-9 and CXCL8 release caused by ECVE prepared from different e-cig brands were found to be similar to, or in excess of, a cigarette smoke extract response. Additionally, MMP-9 and CXCL8 was increased after exposure to ECVE with and without nicotine, suggesting the involvement of other pro-inflammatory constituents.

Here, Ghosh and colleagues investigate the effect of chronic e-cigarette use on the protease-antiprotease balance in the airways of vapers (12). The study recruited never smokers, current tobacco smokers and e-cigarette users (vapers), the latter group including both never-smokers and former tobacco smokers. Protease levels were quantified in bronchoalveolar lavage samples as well as from immune cells stimulated with e-liquid components.

Protease levels were measured using Western blotting and activity by hydrolysis of peptide-based substrates (+/- protease class inhibitors), with gelatin zymography also used to assess the activity of MMP-2 and MMP-9. Importantly, serum nicotine, cotinine and hydroxycotinine were measured to confirm tobacco/vape use; previous work by these authors reported a significant increase in the levels of nicotine and other metabolites in vapers serum, similar to levels observed in cigarette smokers (13).

Western blot analysis showed NE and MMP-2/9 to be significantly elevated in smokers and vapers (including those who reported no prior cigarette use) compared to non-smokers. In addition, relevant protease inhibitors were measured: alpha 1 antitrypsin (AAT), secretory leukocyte proteinase inhibitor (SLPI), TIMP-1 and -2. Similar increases were not observed in protease inhibitor levels indicating a net potential increase in proteolysis.
The number of proteases investigated was then expanded for a spectrofluorimetric analysis, which included other enzymes associated with airway pathophysiology: serine (plasmin, trypsin-like and chymotrypsin-like), cysteine (cathepsin B, S/L and K) and metalloproteases (MMPs 3 and 12). Of these only NE and MMP-2/-9 were upregulated in both smokers and vapers; with cathepsin B increased only in the smokers samples tested. Work on immune cells was conducted using treatments of nicotine and/or a solution comprised of e-cigarette components (3.3 mM nicotine +/- 3% PG/VG (propylene glycol/vegetable glycerine)) with results showing an increase in NE independent of PG/VG. Of note, mannitol was included as a control for potential increases in osmotic stress caused by PG/VG but no effect on NE levels was observed. The increase in NE was subsequently shown to be associated with a rise in cytosolic calcium levels in response to nicotine; earlier studies by the same group found increases in intracellular Ca$^{2+}$ in HEK293T cells exposed to cigarette smoke and the tobacco smoke metabolites 1-NH$_2$-naphthalene, formaldehyde, nicotine, and nicotine-derived nitrosamine ketone (14).

A strength of the study was the robustness of their protease analyses and the measurement of nicotine and its metabolites cotinine and hydroxycotinine in serum, BAL and sputum samples which confirmed the tobacco/vape use of each participant. This was also ensured that a physiologically relevant concentration of nicotine was used to treat neutrophils and alveolar macrophages. A limitation of the study however, is the relatively small numbers recruited to each group and the fact that the vaper group also comprised former tobacco smokers. Furthermore, an inherent problem for researchers investigating the effect of e-cigarettes is the vast array of products and devices on offer which makes it difficult to standardise exposure. The extensive number of formulations and flavourings further increases the level of complexity. A recent study reports e-cigarette products to have an average of 6.2 (SD = 3.6) flavouring chemicals with the sweetest flavours having the greatest number; 21% of products
tested contained flavouring chemicals with potential risk of inhalation toxicity (benzyl alcohol, benzaldehyde, vanillin); other toxicants such as acrolein and diacetyl were also detected and measurable levels of tobacco-specific nitrosamines (TSNAs), an important groups of carcinogens in tobacco products, were present in 70% of tested products (15). The full significance of the inhalation of these complex mixtures of components on the protease-antiprotease balance, the proteome, lung tissue injury and chronic airways disease progression in general will be difficult to determine.

The overall conclusions of the paper that NE and MMP2/9 levels are elevated in vapers, consistent with that seen in smokers, and that this protease imbalance has the potential to increase overall proteolysis in the lung makes a further contribution to the field and lends support to the argument that vaping is not any safer than tobacco smoking. Given that the link between dysregulated proteolysis and lung disease is well-established, coupled with the worrying trend of young, previous non-smokers being attracted to vaping, the possible risk of another generational wave of chronic lung disease in the foreseeable future, must be considered.
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