ST45 Biological and proteomic changes in C57BL/6 mice after 10-wk of inhalation of cigarette smoke and electronic cigarette aerosol

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2022 CORESTA CONGRESS ONLINE

25 OCTOBER 2022 (13:45-14:00)

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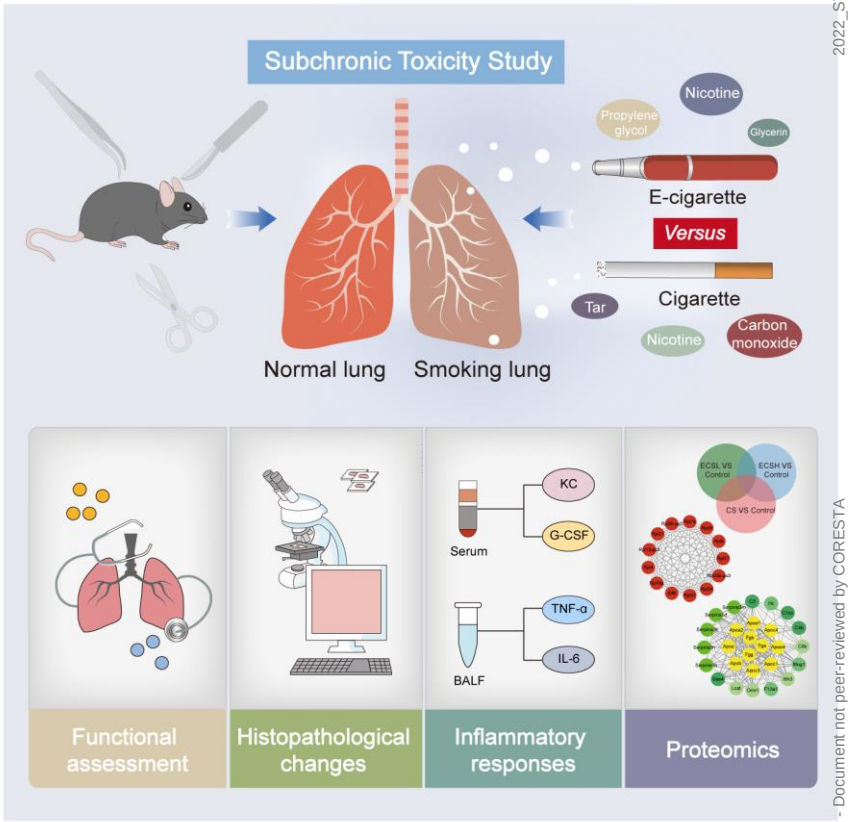
01 Background

- Despite the growing body of evidence supporting the relative safety of e-cigarettes (EC) over smoking, more studies comparing toxicology between e-cigarette aerosol and cigarette smoke (CS) in terms of proteomics are needed

- We standardized nicotine dosage levels in e-cigarette and cigarette exposure and studied the effects using a comprehensive toxicological approach involving physiology, pathology, and proteomics

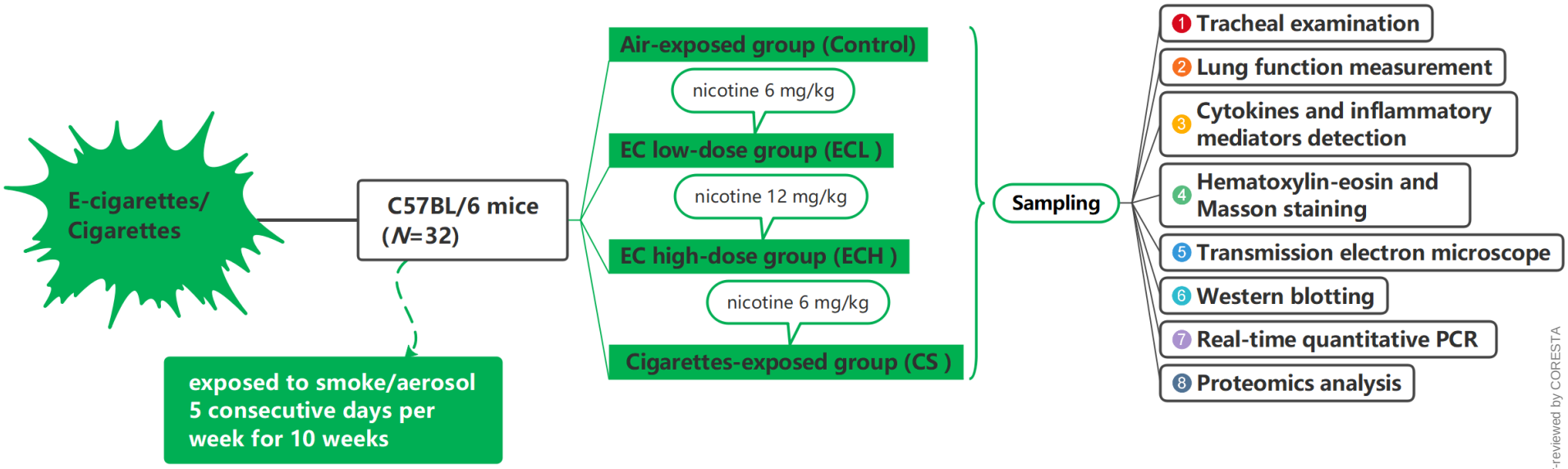
- Using this systemic toxicology assessment framework, we aimed to compare differences in the complex biological responses induced by EC and CS



Graphical illustration of the study design

[1]dependent activation of trpv1 and trpa1 channels through metabolites of cyclooxygenase and 12-lipoxygenase. Respir Res 20(1):110
[2] Beaglehole R, Bates C, Youdan B, Bonita R (2019) Nicotine without smoke: Fighting the tobacco epidemic with harm reduction. Lancet 394(10200):718-720

02 Experimental Methods

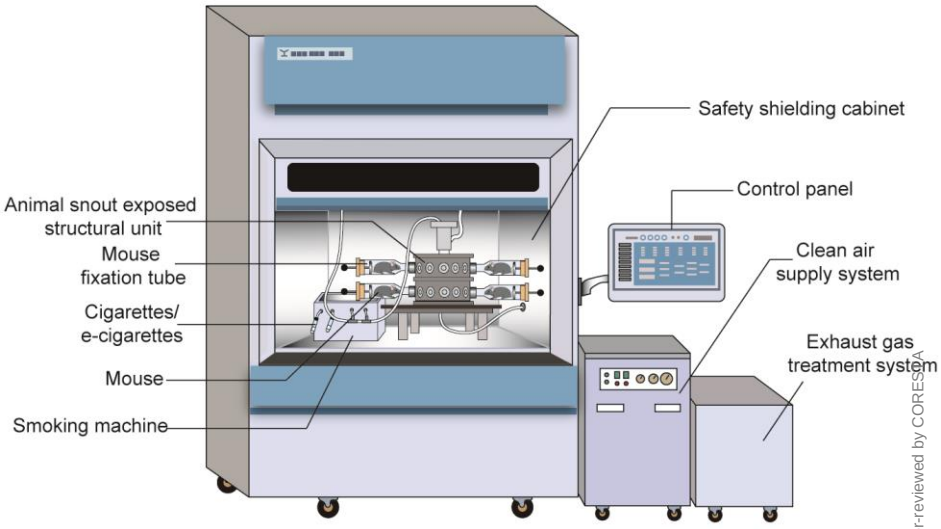


E-cigarettes: Watermelon-flavored closed pod e-cigarettes “Fresh Red” (RELX, China; 3 % nicotine; power, 6.5w)

Cigarettes: Commercially available brand from China; pack labelled: 10 mg tar, 1.0 mg nicotine per cigarette

02 Experimental Methods

- Both the smoking machine and the animal exposure chamber were housed in a safety shielded cabinet
- The smoking machine delivered the smoke/aerosol to the exposure chamber following a standard protocol: 3-s puff, 27-s interval, 55 mL puff volume.
- Mice were kept in the fixation tubes connected to the exposure units and received continuous air or air mixed with emissions from EC or CS.
- The device was also equipped with a clean air supply system and an exhaust gas system.

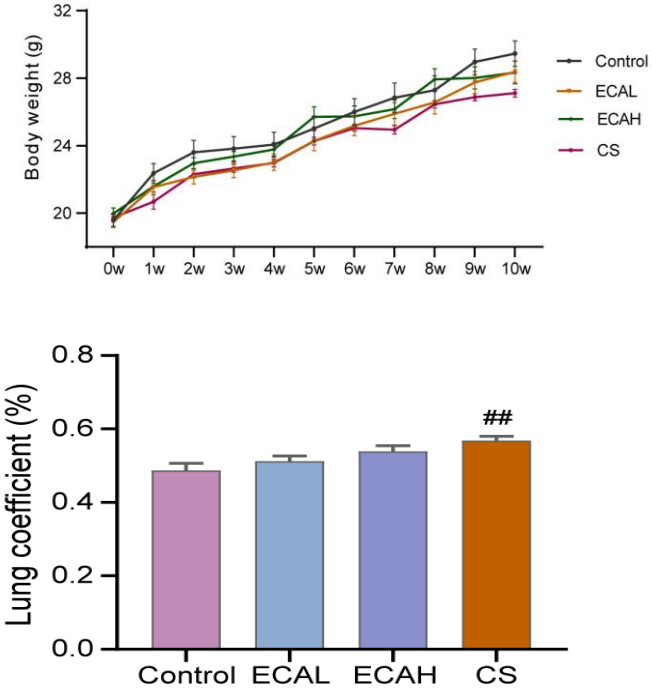


The smoking machine and animal nose-mouth inhalation exposure device

[3] Dusautoir R, Zarcone G, Verriele M, et al. (2021) Comparison of the chemical composition of aerosols from heated tobacco products, electronic cigarettes and tobacco cigarettes and their toxic impacts on the human bronchial epithelial beas-2b cells. J Hazard Mater 401:123417
[4] Drope J, Cahn Z, Kennedy R, et al. (2017) Key issues surrounding the health impacts of electronic nicotine delivery systems (ends) and other sources of nicotine. CA Cancer J Clin 67(6):449-471 https://doi.org/10.3322/caac.21413

03 Results 1 - The variation of body weight and lung coefficients after exposure

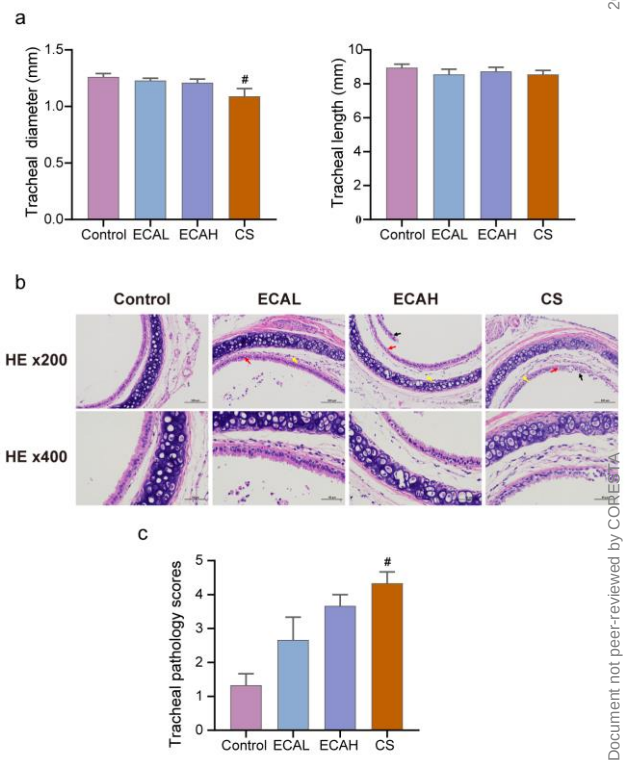
- Before the inhalation study, the mice in each group had a homogeneous weight distribution.
- Mice exposed to EC and CS for 10-wks gained slower bodyweight increases relative to Control, especially the mice in CS（$P > 0.05$）
- CS exposure increased lung coefficients compared to Control.



The Body weight and lung coefficients post exposure

03 Results 2 - Morphological and histological changes of tracheal after exposure

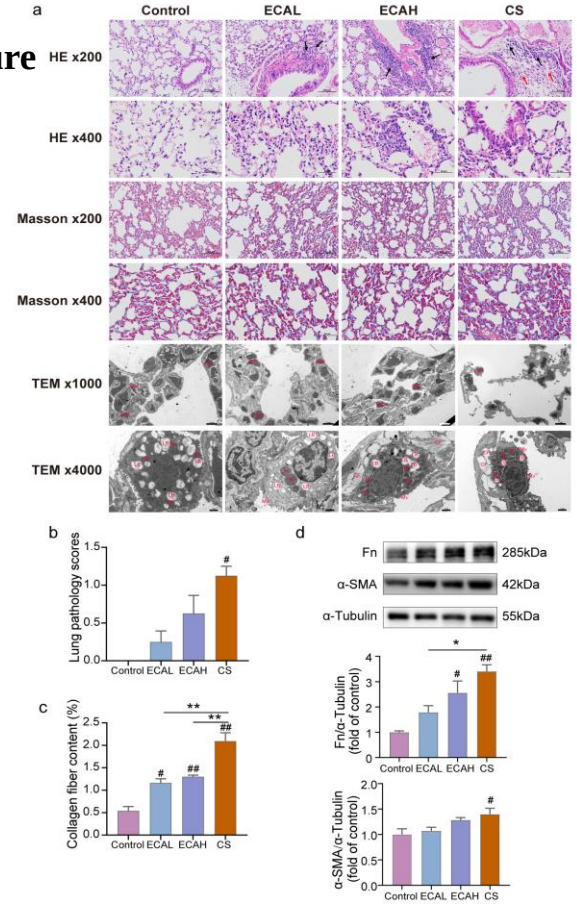
- Tracheal changes in mice the exposure were measured, which showed that CS causes a smaller tracheal diameter but no change in length compared to Control. EC didn't cause changes
- The HE staining revealed that Control mice had intact tracheal mucosa epithelium covered with closely arranged epithelial cells. EC exposures resulted in a small amount of inflammatory cells from the tracheal lumen and submucosa, epithelial cell swelling, and cytoplasmic vacuolization. After CS exposure, epithelial cell detachment and swelling were significantly increased, and cilia were sparse. The submucosa was infiltrated with inflammatory cells and the cytoplasm was vacuolated.
- The pathology score in CS was higher than that in Control, while EC was no statistical difference. E-cigarette aerosol showed less pathological damage to the trachea than cigarette smoke at the same or even 2-fold dose



The morphological and histological structure of trachea

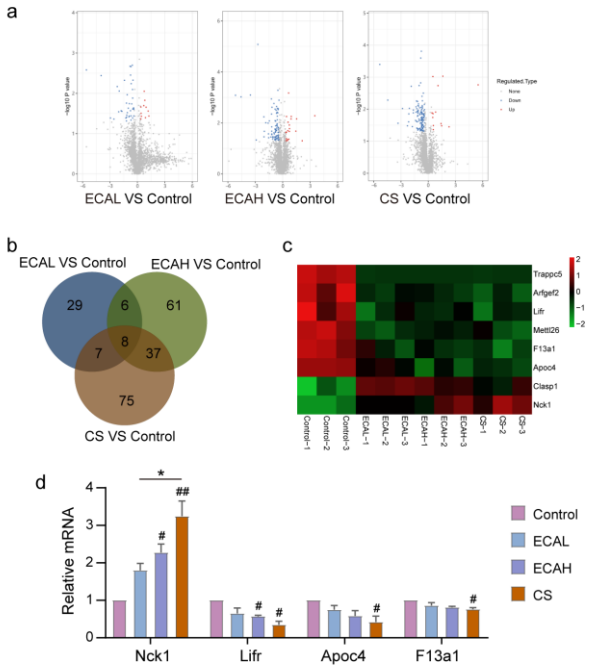
03 Results 3 - Morphological and histological changes of lung after exposure

- Compared to Control, mice in EC-Low had a small infiltration of inflammatory cells around the bronchi (black arrows). However, both EC-High and CS exposure resulted in increased focal infiltration of perivascular inflammatory cells (black arrows). CS additionally exhibited mild hemorrhage around alveoli and blood vessels (red arrows)
- From the pathology scores, CS exposure resulted in higher scores compared to Control, with no significant effect of EC exposure. Masson staining results revealed that both CS and EC exposure resulted in an increase in the percentage area of collagen compared to Control. However, the collagen area was relatively lower in the EC compared to CS
- The expressions of Fn and α-SMA were significantly increased in lung tissue after CS and EC. However, at the same dose as CS, the fold change of Fn and α-SMA expression in EC was lower
- TEM analysis coincided with the observation of HE and Masson staining. The damage to lung pathology was more pronounced with CS compared to that of EC

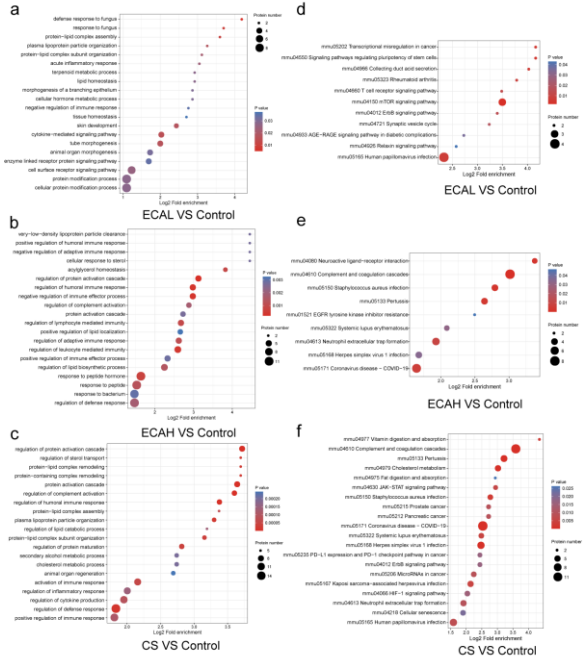


Histopathological changes and scores.

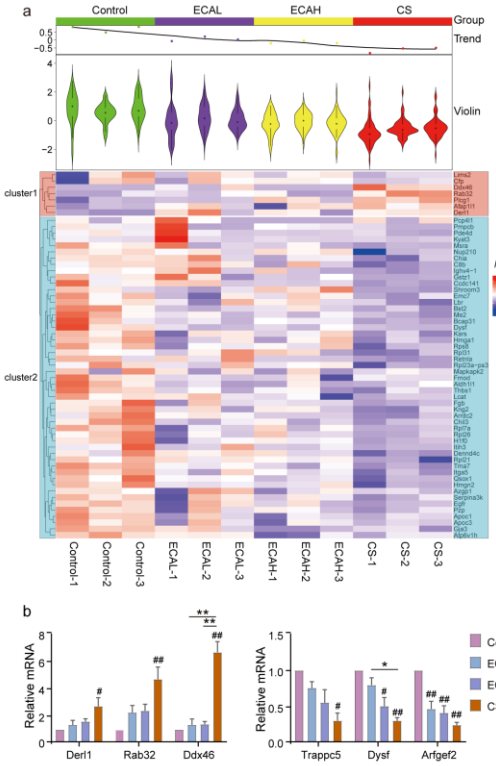
03 Results 4 - Proteomic analysis of lung tissue



Analysis of DEPs in lung tissue.



GO enrichment and KEGG pathway analysis



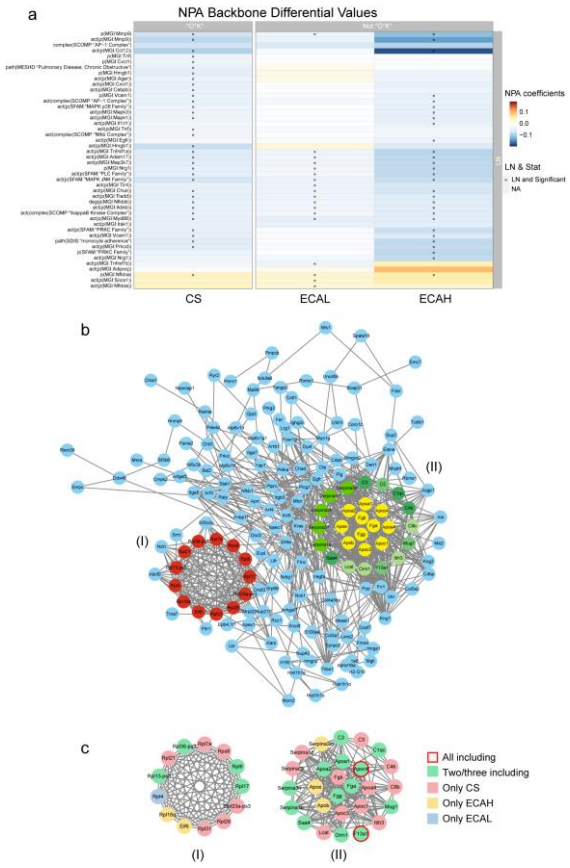
Violin plots of DEPs

03 Results 4 - Proteomic analysis of lung tissue

- Proteomic data quality analysis, 3658 proteins were identified.

Proteins（N）	Increase	Decrease
EC-low	11	39
EC-high	21	91
CS	13	114

- Venn diagrams show that among these significantly different proteins, 8 proteins overlap(Trappc5, Arfgef2, Lifr, Mettl26, F13a1, Apoc4, Clasp1, and Nck1).
- The mRNA expression levels of the overlap proteins (*Nck1*, *Lifr*, *Apoc4*, *and F131)*, the most significant changes were observed in the CS group compared to the control group, followed by EC-High. The changes in EC-Low were not significant



NPA analysis and PPI analysis

04 Conclusions

1. EC showed lower respiratory effects, decreased inflammatory responses, and less elevation of lung fibrosis indicators than cigarette smoke at the same nicotine dose.

2. Proteomic analysis revealed that compared to CS, EC aerosol exposure resulted in fewer differentially expressed proteins and a smaller amount of disruption of inflammatory networks.

3. CS and EC had an observable effect on ribosomal proteins and complement system proteins. CS exposure in particular may lead to impaired ribosome function, thereby affecting the expression of other proteins and leading to reduced immunity

4. Our findings indicated that EC aerosol appeared to be less hazardous to the respiratory system than that CS at the same nicotine dose, providing further evidence to support human studies for e-cigarettes' relative safety.

[5] Lee KM, Hoeng J, Harbo S, et al. (2018) Biological changes in c57bl/6 mice following 3 weeks of inhalation exposure to cigarette smoke or e-vapor aerosols. Inhal Toxicol 30(13-14):553-567 https://doi.org/10.1080/08958378.2019.1576807
[6] Lee PN, Fry JS, Gilliland S, 3rd, Campbell P, Joyce AR (2022) Estimating the reduction in us mortality if cigarettes were largely replaced by e-cigarettes. Arch Toxicol 96(1):167-176 https://doi.org/10.1007/s00204-021-03180-3

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Thanks

05 / Q&A session

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