The Effects of Cigarette Smoke on SARS-CoV-2 Infection in Human Gingival Epithelial Cells

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Objectives

Our objective was to examine the effects of cigarette smoke on SARS-CoV-2 infection in human gingival epithelial cells (GECs). We wanted to better understand the mechanisms involved in SARS-CoV-2 infection in cells of the oral cavity, which may suggest therapeutic interventions for preventing viral infection and transmission.

Introduction

A large body of evidence shows the harmful effects of cigarette smoke on oral and systemic health. A meta-analysis of 11,322 COVID-19 patients showed active smokers were twice as likely to develop severe COVID-19, compared with non-smokers [1].

Molecules from cigarette smoke can stimulate the aryl hydrocarbon receptor (AhR), which is a ubiquitously-expressed transcription factor activated by a variety of agonists. A xenobiont, 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) is the best known AhR ligand. Since ACE-2 mediates SARS-CoV-2 infection, and because ACE2 expression was significantly increased in GECs after treatment with TCDD or CSC, we hypothesize that CSC might favor SARS-CoV-2 infection in GECs.

Methods/ Results

Human gingival epithelial cells (GECs) were treated with or without cigarette smoke condensates CSC at different concentrations as indicated, or with TCDD(10 nM) for 24 h, prior to infection with SARS-CoV-2 pseudotyped GFP-tagged lentivirus for an additional 24 h. Viral infection was measured by immunofluorescence microscopy, and fluorescence intensity was measured using ImageJ software.

siRNA was used to deplete AhR in GECs before treating the cells with TCDD or CSC, and infecting with the SARS-CoV-2 Pseudovirus as described above.

siRNA was used to deplete AhR in GECs before treating the cells with TCDD or CSC. Next, the cells were treated with or without cigarette smoke condensate (10 ug/mL) for 24 hours. Next, the cells were infected with SARS-CoV-2 pseudovirus. Using cells transfected with siRNA control, we observed high levels of SARS-Cov-2 pseudovirus internalization after CSC treatment.

Results

Cigarette smoke condensates increase SARS-CoV-2 pseudovirus internalization in gingival epithelial cells

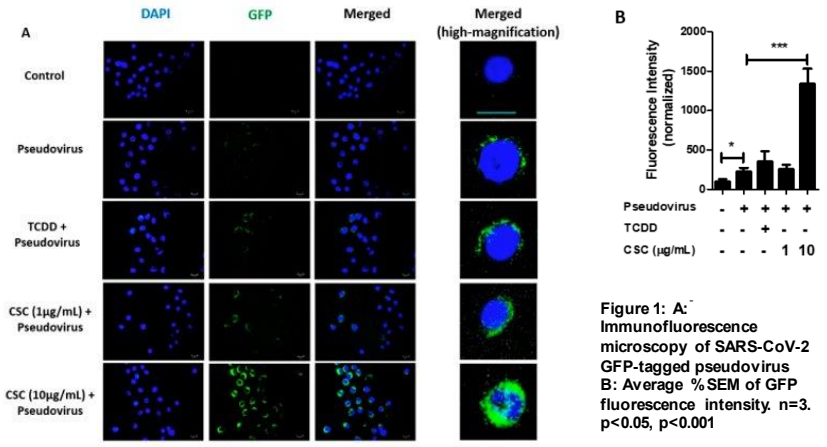

Figure 1: A: Immunofluorescence microscopy of SARS-CoV-2 GFP-tagged pseudovirus B: Average %SEM of GFP fluorescence intensity. n=3. p<0.05, p<0.001

Cigarette smoke condensates promote SARS-CoV-2 pseudovirus internalization via AhR in gingival epithelial cells

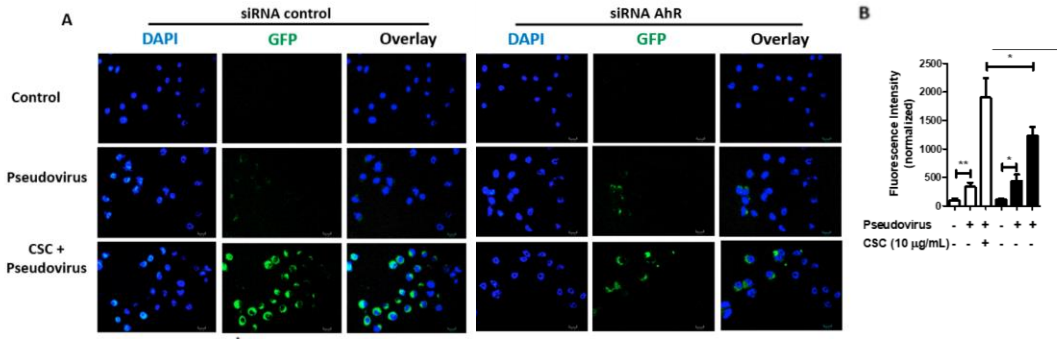

Figure 2:
A:Immunofluorescence microscopy of SARS-CoV-2 GFP-tagged pseudovirus
B: Average %SEM of GFP fluorescence intensity. n=3. p<0.05, p<0.001

Conclusion

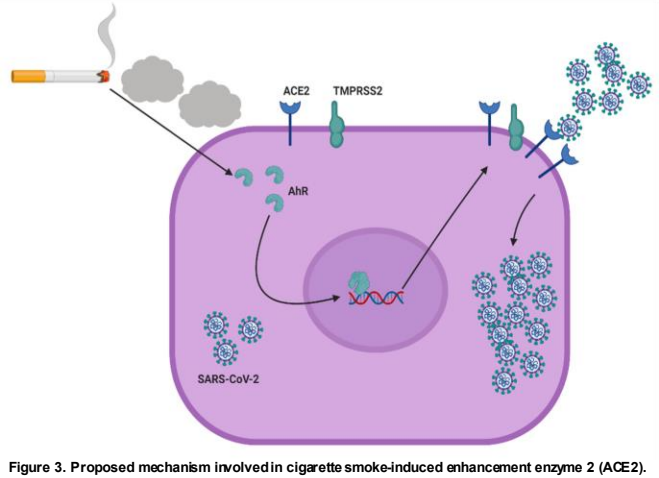

Figure 3. Proposed mechanism involved in cigarette smoke-induced enhancement enzyme 2 (ACE2).

- GECs pretreated with TCDD or CSC at 1µg/ml showed no change in SARS-CoV-2 pseudovirus internalization compared with infection of untreated GECs
- GECs pretreated with CSC at 10 µg/mL showed a significant increase in SARS-CoV-2 pseudovirus infection compared with infection of untreated control GECs.
- GECs transfected with siRNA against AhR and treated with CSC showed lower levels of SARS-Cov-2 pseudovirus internalization, compared to GECs transfected with siRNA control and treated with CSC.
- Cigarette smoke activates aryl hydrocarbon receptor (AhR) in human gingival epithelial cells, which increases the surface levels of angiotensin-converting enzyme 2 (ACE2), and facilitates SARS-Cov-2 infection.

References

1. Gulsen, A. et al. The Effect of Smoking on COVID-19 Symptom Severity: Systematic Review and Meta-Analysis. *Pulm Med.* **2020**, *2020*, 7590207.

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