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Brief Report
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Brief Report

Instigators of COVID-19 in Immune Cells Are Increased in Tobacco Cigarette Smokers and Electronic Cigarette Vapers Compared With Nonsmokers

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Abstract

Introduction: The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the virus responsible for the COVID-19 pandemic, gains entry into the host cell when its Spike protein is cleaved by host proteases TMPRSS2 and furin, thereby markedly increasing viral affinity for its receptor, angiotensin-converting enzyme-2 (ACE2). In rodent and diseased human lungs, tobacco cigarette (TCIG) smoke increases ACE2, but the effect of electronic cigarette vaping (ECIG) is unknown. It is unknown whether nicotine (in both TCIGs and ECIGs) or non-nicotine constituents unique to TCIG smoke increase expression of key proteins in COVID-19 pathogenesis.

Methods: Immune ($CD45^+$) cells collected before the pandemic in otherwise healthy young people, including TCIG smokers ($n = 9$), ECIG vapers ($n = 12$), or nonsmokers (NS) ($n = 12$), were studied. Using flow cytometry, expression of key proteins in COVID-19 pathogenesis were compared among these groups.

Results: TCIG smokers and ECIG vapers had similar smoking or vaping burdens as indicated by similar plasma cotinine levels. TCIG smokers compared with NS had a significantly increased percentage of cells that were positive for ACE2 (10-fold, $p < .001$), TMPRSS2 (5-fold, $p < .001$), and ADAM17 (2.5-fold, $p < .001$). Additionally, the mean fluorescence intensity (MFI) consistently showed greater mean ACE2 (2.2-fold, $p < .001$), TMPRSS2 (1.5-fold, $p < .001$), furin (1.1-fold, $p < .05$), and ADAM17 (2-fold, $p < .001$) in TCIG smokers compared with NS. In ECIG vapers, furin MFI was increased (1.15-fold, $p < .05$) and TMPRSS2 MFI tended to be increased (1.1-fold, $p = .077$) compared with NS.

Conclusions: The finding that key instigators of COVID-19 infection are lower in ECIG vapers compared with TCIG smokers is intriguing and warrants additional investigation to determine if switching to ECIGs is an effective harm reduction strategy. However, the trend toward increased proteases in ECIG vapers remains concerning.

Implications: (1) This is the first human study to report a marked increase in proteins critical for COVID-19 infection, including ACE2, TMPRSS2, and ADAM17, in immune cells from healthy tobacco cigarette smokers without lung disease compared with e-cigarette vapers

and nonsmokers. (2) These findings warrant additional investigation to determine whether switching to electronic cigarettes may be an effective harm reduction strategy in smokers addicted to nicotine who are unable or unwilling to quit. (3) The increase in proteases in electronic cigarette vapers remains concerning.

Introduction

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the virus responsible for COVID-19, has infected almost 180 million people worldwide, resulting in almost 4 million deaths in the ongoing COVID-19 pandemic.[1] SARS-CoV-2 gains cell entry when its spike protein binds to the host receptor, angiotensin-converting enzyme-2 (ACE2).[2] Before the spike protein can bind to its receptor, it is cleaved or "primed" by host proteases such as TMPRSS2 and furin, thereby increasing affinity for the host receptor ACE2 by 20-fold.[3] Beyond this, very little is known about mechanisms of virulence of the SARS-CoV-2 and the factors increasing vulnerability to severe infection. The role of smoking has been controversial. Paradoxically, unlike virtually any other respiratory infection, tobacco cigarette (TCIG) smokers have been *underrepresented* in reports of patients hospitalized with COVID-19.[4] This has led to speculation that something in TCIG smoke, specifically nicotine which has anti-inflammatory effects, is protective.[5] On the other hand, TCIG smoking has been identified as a risk factor for progression to more severe disease and death in those already hospitalized with COVID-19.[6]

ACE2, the receptor for SARS-CoV-2, is ubiquitous in human tissues, present in the lung, gut, kidney, heart, and other tissues, as well as in endothelial, vascular smooth muscle, and immune cells.[3] This ubiquity may explain, in part, the diverse presentations of COVID-19, including the cytokine release syndrome that is responsible for many deaths. Importantly, in rodent and diseased human lungs, TCIG smoking is associated with increased ACE2 expression.[7] It is unknown if increased ACE2 is attributable to the underlying TCIG-associated lung disease or if TCIG smoke itself increases ACE2 expression, even in the absence of lung disease. Electronic cigarette (ECIG) vaping is increasing among smokers trying to quit, but also among young nonsmokers.[8,9] Emissions from ECIGs contain far fewer toxicants, which, if detected at all, are present in concentrations orders of magnitude lower compared with emissions from TCIGs—except for one, nicotine. Levels of cotinine, the metabolite of nicotine, are comparable in smokers and vapers.[10] ECIG vaping has variably been associated with increased risk,[11] and no increased risk,[12] for COVID-19 diagnosis.

We reasoned that studies in healthy young people without lung disease, who only smoke TCIGs or only vape ECIGs, would clarify this issue whether TCIG smoking alone, in the absence of lung disease, increases expression of key instigators of COVID-19 infection, such as ACE2 and several proteases. Moreover, these findings would provide insights into the relative effects of TCIG smoking and ECIG vaping, thereby providing valuable and urgently needed information to inform public health strategies to decrease vulnerability to COVID-19 infection.

Methods

The data that support the findings of this study are available from the corresponding author on reasonable request. Accessible peripheral blood mononuclear cells (PBMCs) collected in 2019, before the pandemic, from 33 (19 males) healthy young (mean age of 24 years old) people, including 9 chronic TCIG smokers, 12 chronic ECIG vapers, and 12 nonsmokers,[13] were available for study. Baseline characteristics of participants including age, sex, race, and educational level have been published and did not differ among groups.[13] Seven of the 12 ECIG vapers were former TCIG smokers, all quitting more than 2 years before the study. None were current TCIG smokers. Importantly, TCIG smokers and ECIG vapers had similar current smoking and vaping burdens based on plasma cotinine levels, and all had refrained from smoking 12 hours before the study session.[13] In thawed PBMCs, we used multicolor flow cytometry[14] to determine protein levels of ACE2, TMPRSS2, furin, and ADAM17, the protease responsible for ACE2 shedding and which promotes inflammation through dysregulation of interleukin-6,[15] a major correlate of COVID-19 mortality.[16]

To confirm whether the expression of these key proteins in accessible PBMCs has relevance to other target tissues for the SARS-CoV-2 virus, expression of ACE2, TMPRSS2, furin, or ADAM17 in gut tissue, a target tissue for SARS-CoV-2 infection that has the same embryogenesis as lung tissue,[17] was correlated with PBMC expression.[3] Gut epithelial tissue was available from colonoscopies performed in 11 otherwise healthy nonsmokers (10 males, mean age 52 years).

This is an exploratory, hypothesis-generating small study, and we did not adjust for multiple comparisons. The experimental protocols were approved by the Institutional Review Board, and all participants provided written informed consent.

Results

Percentage of Immune ($CD45^+$) Cells Positive for Key Proteins in COVID-19 Infection

TCIG smokers had a strikingly increased percentage of $CD45^+$ immune cells that were positive for ACE2, TMPRSS2, and ADAM17 compared with ECIG vapers and nonsmokers (Supplementary Figure 1A–H). In contrast, the percentage of immune cells that were positive for ACE2, TMPRSS2, and ADAM17 in ECIG vapers and nonsmokers were virtually identical. The percentage of $CD45^+$ cells positive for furin was not different among the three groups.

Mean Fluorescence Intensity of Key Proteins in COVID-19 Infection

Between group comparisons of the mean fluorescence intensity (MFI) consistently demonstrated greater ACE2, TMPRSS2, and ADAM17 levels in $CD45^+$ cells from TCIG smokers compared with ECIG vapers and nonsmokers (Supplementary Figure 1I–D). Furin MFI in $CD45^+$ cells from TCIG smokers was also higher compared with nonsmokers (Supplementary Figure 1D). In $CD45^+$ cells from ECIG vapers, furin MFI was increased, whereas TMPRSS2 MFI tended ($p = .077$) to be increased compared with nonsmokers (Supplementary Figure 1K and L).

Expression of each these key proteins in gut cells was positively and consistently correlated with respective protein levels in PBMCs (Supplementary Figure 2A–F).

Discussion

To the best of our knowledge, this is the first human study to report a marked increase in ACE2, TMPRSS2, and ADAM17 in TCIG smokers compared with ECIG vapers and nonsmokers. The increases in key instigators of COVID-19, including ACE2, in otherwise healthy young TCIG smokers in the absence of clinical lung disease demonstrates that TCIG smoke itself induces upregulation of these proteins. Importantly, this increase in ACE2 and proteases levels was not induced by a differences in the burden of TCIG smoking and ECIG vaping, since plasma cotinine levels were not different,[13] implicating non-nicotine toxicants in TCIG smoke in inducing expression of ACE2 and proteases. Although this increased expression may be reversible, since many of our ECIG vapers were former TCIG smokers, this remains speculative. Furthermore, ECIG vapers had a small increase in TMPRSS2 and furin in immune cells compared with nonsmokers, a finding that deserves further study.

Although the role of TCIG smoking in SARS-CoV-2 infection remains controversial,[4,6] our study contributes to the evidence that smoking is harmful. Importantly, in TCIG smokers, the upregulation of (1) ACE2, the receptor for SARS-CoV-2; (2) TMPRSS2 and furin, which modify the spike protein enhancing affinity 20-fold for ACE2[18]; and (3) ADAM17, which may worsen lung injury in COVID-19 by promoting inflammation through ACE2 shedding and dysregulation of IL-6,[15] a correlate of mortality in COVID-19,[16] provides insight into the mechanisms whereby TCIG smoking may increase vulnerability to severe COVID-19, even in healthy young smokers.

Our study is a small study. Nonetheless, using two different and complementary measures, we were able to find a consistent increase in cellular ACE2, TMPRSS2, and ADAM17 in TCIG smokers compared with nonsmokers. We studied readily accessible PBMCs instead of more commonly infected, but comparatively inaccessible, cells such as lung epithelial cells. We reasoned that PBMCs were reasonable surrogates since we found that expression of these key proteins in PBMCs was positively correlated with respective protein levels in gut cells, and gut and lung tissue have the same embryogenesis. Nonetheless, confirmation of these findings in additional participants and tissues is warranted.

In conclusion, the finding that key instigators of COVID-19 in immune cells are lower in chronic ECIG vapers compared with TCIG smokers is intriguing and warrants additional investigation to determine whether switching to ECIGs may be a harm-reduction strategy.[19] However, the trend toward increased proteases in ECIG vapers remains concerning.

Supplementary Material

A Contributorship Form detailing each author's specific involvement with this content, as well as any supplementary data, are available online at https://academic.oup.com/ntr.

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Declaration of Interests

None declared.

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