The Pathophysiology of COVID-19 and SARS-CoV-2 Infection

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Elevated FiO₂ increases SARS-CoV-2 co-receptor expression in respiratory tract epithelium

Myti D, Gunjak M, Casado F, Khaghani Raziabad S, Nardiello C, Vadász I, Herold S, Pryhuber G, Seeger W, Morty RE. Elevated FiO₂ increases SARS-CoV-2 co-receptor expression in respiratory tract epithelium. Am J Physiol Lung Cell Mol Physiol 319:L670–L674, 2020. First published September 2, 2020; doi:10.1152/ajplung.00345.2020.—The severity of coronavirus disease 2019 (COVID-19) is linked to an increasing number of risk factors, including exogenous (environmental) stimuli such as air pollution, nicotine, and cigarette smoke. These three factors increase the expression of angiotensin I converting enzyme 2 (ACE2), a key receptor involved in the entry of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)—the etiological agent of COVID-19—into respiratory tract epithelial cells. Patients with severe COVID-19 are managed with oxygen support, as are at-risk individuals with chronic lung disease. To date, no study has examined whether an increased fraction of inspired oxygen (FiO₂) may affect the expression of SARS-CoV-2 entry receptors and co-receptors, including ACE2 and the transmembrane serine proteases TMPRSS1, TMPRSS2, and TMPRSS11D. To address this, steady-state mRNA levels for genes encoding these SARS-CoV-2 receptors were assessed in the lungs of mouse pups chronically exposed to elevated FiO₂, and in the lungs of preterm-born human infants chronically managed with an elevated FiO₂. These two scenarios served as models of chronic elevated FiO₂ exposure. Additionally, SARS-CoV-2 receptor expression was assessed in primary human nasal, tracheal, esophageal, bronchial, and alveolar epithelial cells, as well as primary mouse alveolar type II cells exposed to elevated oxygen concentrations. While gene expression of ACE2 was unaffected, gene and protein expression of TMPRSS11D was consistently upregulated by exposure to an elevated FiO₂. These data highlight the need for further studies that examine the relative contribution of the various viral co-receptors on the infection cycle, and point to oxygen supplementation as a potential risk factor for COVID-19.

COVID-19; FiO₂; hyperoxia; SARS-CoV-2; TMPRSS

INTRODUCTION

The world currently finds itself in the grip of the coronavirus disease 2019 (COVID-19) pandemic caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (18). A number of risk factors for severe COVID-19 have been identified, including advanced age, as well as comorbidities including hypertension, obesity, malignancy, and chronic lung disease (11). Risk factors could also include stimuli that modify susceptibility to SARS-CoV-2 infection, for example, by modulating the expression of receptors that mediate viral entry into cells. Endogenous physiological modulators of viral entry receptors such as estrogen (16) and interferon (19) have recently been identified. However, exogenous (environmental) modulators of virus receptor expression may represent hitherto unrecognized risk factors for COVID-19. Indeed, air pollution (3) as well as cigarette smoke (8) and nicotine (7) have recently been proposed to increase the risk of severe COVID-19 by driving expression of the SARS-CoV-2 entry receptor angiotensin I converting enzyme 2 (ACE2) (5).

COVID-19 patients with acute respiratory failure are managed with supplemental oxygen (18), and elderly patients and infants born preterm with restrictive and obstructive lung disease may use home oxygen therapy. This raises the question of whether an elevated FiO₂ might alter the expression of the virus entry receptor ACE2, as well as the virus co-receptors that facilitate cellular entry of SARS coronaviruses, including the serine peptidases TMPRSS1, TMPRSS2, and TMPRSS11D (4, 10). The full spectrum of SARS-CoV-2 receptors has not been definitively clarified, and a growing number of candidate receptors continue to be profiled in the epithelium of the entire respiratory tract (1, 17). To answer the question of whether an elevated FiO₂ might impact the regulation of virus receptor genes, the expression of SARS-CoV-2 entry receptors and co-receptors was assessed in lung tissue from preterm-born infants with bronchopulmonary dysplasia (BPD) managed chronically with elevated FiO₂, or from mouse pups exposed to elevated FiO₂ for protracted periods in an animal model of BPD. It is critical to emphasize that this study is neither a BPD study, nor a study on addressing COVID-19 in infants (9). Rather, the clinical and experimental BPD models served purely as models of chronic supplemental oxygen exposure. This report should not be misunderstood as a pediatric study. Newborn mice were employed in the present study because newborn mice tolerate protracted periods of exposure to high FiO₂, whereas adult mice do not and rapidly succumb to lethal acute lung injury. The expression of entry receptors and co-receptors was also assessed in adult...
mouse and adult human epithelial cells originating from different regions of the respiratory tract between the nose and the alveoli, as well as the esophagus, which were exposed in vitro to elevated oxygen concentrations.

MATERIALS AND METHODS

Mouse tissue. In an experimental animal model of BPD [Institutional Animal Care and Use Committee (IACUC) approval for RNA profiling of mouse lung tissues under approval references B2/277 and B2/351]. Newborn mouse pups inhaled an FiO2 of 0.21 or 0.85 from the day of birth to post-natal day (P)14 (12).

Human tissue. Harvesting of human lung tissue from BPD patients [x birth wt 706 ± 222 g; 6 male/4 female; x duration FiO2 >0.5, 29 day (range 5–96 days)] and term infant controls (x birth wt 2,033 ± 1,356 g; 2 male/6 female; no oxygen supplementation) without lung disease was approved by the Institutional Review Board of the University of Rochester (2). Written informed consent was obtained from guardians.

Human and mouse lung epithelial cells. Mouse alveolar epithelial type II cells were isolated from female C57BL/6J mice (3–6 mo of age) as described previously (14). Human airways and lung epithelial cells were purchased from Cell Biologics (Planegg, Germany): tracheal (H-6033), esophageal (H-6046), bronchial (H-6033B), and alveolar (H-6053). Human nasal epithelial cells were purchased from Promocell (C-12620). The sexes of the donors of the human respiratory tract cells were not disclosed by the vendor. Cells were not passaged, and were cultured per manufacturer’s recommendations, and maintained on air-liquid interfaces in a 21% or 85% O2 atmosphere (14).

Real-time RT-PCR profiling of gene expression. The abundance of mRNA transcripts was screened by real-time RT-PCR (14) using the primer pairs (5'-3'): human ACE2 (ACAGTCCACACTTGCCCAAAT, TGAGAGCACTGAAGAGCCATT), TMPRSS1 (CTCTGCCCCTCACAGAATACAT, GACATCATGGTGATATTGGGGA), TMPRSS2 (CAATGCTCAAACCTCTCGGGGT, AACAACCCCATCTCCTGCTC), TMPRSS11D (CAGACTTGGAAGCCACTGGCA, ACTCCACCTTGGGATGTACCTCCA), with POLR2A as reference gene (GCGGAATGGAAGCACGTTAAT, CCCAGCACAAAACACTCCTC); and mouse Ace2 (GCAGATGCTACAACTATAACCG, CCTCCTCACATAGGCATGAAGA), Tmprss1 (TACCTTCCCTTACAGACCTCCAT, CCACTGACTGTGGTACCCCA), Tmprss2 (GCTGTCTTGCTTTGGAGGTTC, GACAATGTGCTACCCCGTCA), Tmprss11d (GGTACAGCTC3P2P2P5P10P14

Fig. 1. Steady-state levels of lung mRNA transcripts encoding severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) entry receptors and co-receptors in experimental animals and clinical subjects chronically exposed to elevated FiO2. A: steady-state mRNA levels were determined by real-time RT-PCR for Ace2, Tmprss1, Tmprss2, and Tmprss11d in lung homogenate cDNA from C57BL/6J mouse pups (n = 5 animals per group) at post-natal day (P)2, 3, 5, 10, and 14, exposed either to room air (FiO2 0.21; yellow bars) or hyperoxia (FiO2 0.85; blue bars) for the first 14 days of post-natal life. B: steady-state mRNA levels were similarly determined for ACE2, TMPRSS1, TMPRSS2, and TMPRSS11D in lung homogenate cDNA from infants without (Ctrl, n = 8 patients; yellow bars) or with bronchopulmonary dysplasia (BPD; n = 10 patients; blue bars). Data reflect mean △Ct ± SD. Pairwise comparisons were made between the 21% O2 and 85% O2 groups by unpaired Student’s t test (A), and between the Ctrl and BPD groups by Mann-Whitney U test (B). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. ACE2, angiotensin I converting enzyme 2; POLR2A/Polr2a, RNA polymerase II subunit A; TMPRSS, transmembrane serine protease.
CGTAACACTCGTG, GCTGGGAGACATACCCTATGGAT), with Polr2a as reference gene (CTAAGGGGCAGCCAAAGAAAC, CATTCAGCATACAATCAGGC). Data are presented as difference in cycle threshold (Ct), \( \Delta \text{Ct} \), given by \( \text{Ct}_{(\text{gene of interest})} - \text{Ct}_{(\text{Polr2a})} \). Fold-change in mRNA abundance was given by fold-change \( \left(\frac{2^{\Delta \text{Ct}}}{2^{\Delta \text{Ct}}}\right) \).

**Immunoblotting.** Protein extracts were prepared as described previously (15), from the neonate mouse lungs and the human tracheal epithelial cells described above. Proteins were resolved on a 12% reducing SDS-PAGE gel, electroblotted to a nitrocellulose membrane. TMPRSS11D was detected with a rabbit anti-human TMPRSS11D (ab127031, Abcam, Cambridge, UK; 1:1,500) which detected both the human and mouse antigen. Two proteins were employed to document loading equivalence: \( \beta \)-actin and \( \beta \)-tubulin. Two different loading controls were employed because \( \beta \)-actin has a similar molecular mass to TMPRSS11D, and loading equivalence was documented on the same membrane. Thus, the TMPRSS11D signal may have affected the \( \beta \)-actin signal. \( \beta \)-Actin was detected with a rabbit anti-human \( \beta \)-actin antibody (4967S, Cell Signaling Technology, Frankfurt am Main, Germany; 1:1,000), and \( \beta \)-tubulin was detected with a rabbit anti-human \( \beta \)-tubulin antibody (ab6046, Abcam, Cambridge, UK; 1:1,000). Immune complexes were detected with a horseradish peroxidase-conjugated goat anti-rabbit IgG (H+L) (31460, ThermoFisher, Waltham, MA; 1:3,000), and visualized using SuperSignal West Femto Maximum Sensitivity Substrate (ThermoFisher, Waltham, MA).

**RESULTS AND DISCUSSION**

Expression of SARS-CoV-2 receptors in experimental and clinical BPD. Exposure of newborn mouse pups to FiO\(_2\) 0.85 increased lung expression of \( Tmprss11d \) 10.5-fold after 2 days, and \( Tmprss11d \) expression remained elevated, up to 40-fold at 10 days (Fig. 1A). By 14 days, \( Ace2 \), \( Tmprss1 \), and \( Tmprss2 \) expression was increased 1.5-, 2-, and 1.5-fold, respectively (Fig. 1A). The increased protein expression of TMPRSS11D in the lungs of mice exposed to FiO\(_2\) 0.85 was validated by immunoblot (see Fig. 3A). In BPD patients that were chronically managed with FiO\(_2\) > 0.5, no changes in \( ACE2 \), \( TMPRSS1 \), and \( TMPRSS2 \) expression were noted. However, a 2.5-fold increase in...
The key question posed in this study: "does increased FiO₂ drive SARS-CoV-2 entry receptor or co-receptor expression?" was addressed using lung tissue from patients with BPD, as well as an experimental animal model of BPD. This was neither a BPD nor a pediatric study. Rather, clinical or experimental BPD was employed as a model of supplemental oxygen support with lengthy use of an FiO₂ of >0.5. The data presented here document that elevated FiO₂ increased expression of TMPRSS2 and TMPRSS11D, which are required for efficient viral entry of SARS-CoV-2 (13). TMPRSS11D in particular has emerged in this study as being oxygen-responsive in both mouse and human airways and lungs. These data are potentially important, given that COVID-19 patients with acute respiratory failure are managed with oxygen support, which might increase viral co-receptor expression in the airways and lung epithelium of affected patients, thus accelerating the infection cycle. High-risk patients such as elderly (or young) patients with chronic lung disease in which home oxygen therapy forms part of routine disease management, or preterm born survivors of BPD discharged on home oxygen, may be similarly affected. These data highlight the need for further studies that examine the relative contribution of the various viral co-receptors on the infection cycle, and point to oxygen supplementation as a potential risk factor for COVID-19.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

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**Figure 3.** Steady-state TMPRSS11D protein expression levels in human tracheal epithelial cells and in the lungs of mice or exposed elevated oxygen levels. A: newborn mouse pups were exposed to either an FiO₂ of 0.21 or 0.85 for the first 14 days of postnatal life, after which lungs were harvested for protein isolation. B: primary human tracheal epithelial cells were cultured on an air-liquid interface and exposed to a 21% O₂ or an 85% O₂ incubator headspace environment, and processed for protein isolation. Protein extracts were resolved on a 12% reducing SDS-PAGE gel, electroblotted to nitrocellulose, and TMPRSS11D was detected by immunoblot. Blots were reprobed for both β-actin (which has the same formula mass as TMPRSS11D), and β-tubulin, which served as controls for loading equivalence. The uncropped blots are provided in Supplemental Fig. S1 (https://doi.org/10.6084/m9.figshare.12855341.v1). TMPRSS, transmembrane serine protease.
REFERENCES

1. Aguiar JA, Tremblay BJ, Mansfield MJ, Woody O, Lobb B, Banerce J, A, Chandiramohan A, Tiessen N, Cao O, Dvorin-Gheva A, Revill S, Miller MS, Carlsten C, Organ L, Joseph C, John A, Hanson P, Austin RC, McManus BM, Jenkins G, Mossman K, Ask K, Dovey AC, Hirota JA. Gene expression and in situ protein profiling of candidate SARS-CoV-2 receptors in human airway epithelial cells and lung tissue. Eur Respir J 56: 2001123, 2020. doi: 10.1183/13993003.01123-2020.

2. Bhattacharya S, Go D, Krenitsky DL, Huyck HL, Solleti SK, Lungen VA, Metlay L, Srisuma S, Wert RE, Mariani TJ, Pryhuber GS. Genome-wide transcriptional profiling reveals connective tissue mast cell accumulation in bronchopulmonary dysplasia. Am J Respir Crit Care Med 186: 349–358, 2012. doi: 10.1164/rccm.201203-0406OC.

3. Frontera A, Cianfanelli L., Viachos K, Landoni G, Cremona G. Severe air pollution links to higher mortality in COVID-19 patients: the "double-hit" hypothesis. J Infect 81: 255–259, 2020. doi: 10.1016/j.jinf.2020.05.031.

4. Heurich A, Hofmann-Winkler H, Gierer S, Liepold T, Jahn O, Pöhlmann S. TMPRSS2 and ADAM17 cleave ACE2 differentially and only proteolysis by TMPRSS2 augments entry driven by the severe acute respiratory syndrome coronavirus spike protein. J Virol 88: 1293–1307, 2014. doi: 10.1128/JVI.02202-13.

5. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Whissel L, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA, Drosten C, Pöhlmann S. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 181: 271–280.e8, 2020. doi: 10.1016/j.cell.2020.02.052.

6. Hou YJ, Okuda K, Edwards CE, Martínez DR, Asakura T, Dinnon KH III, Kato T, Lee RE, Yount BL, Masenkic TM, Chen G, Olivier KN, Ghio A, Tse LV, Leist SR, Gralinski LE, Schäfer A, Deng H, Gilmore R, Nakano S, Liu L, Fulcher ML, Livraghi-Butrico A, Nicely B, Plaisant M, Ansari M, Angelides I, Adler H, Sucre JMS, Taylor CJ, Lin B, Wahagry A, Mitsiatis S, Dwyer DF, Buschle KM, Boyce JA, Barrett NA, Laidlaw TM, Carroll SL, Colonna L, Tkachev V, Peterson CW, Yu A, Zheng HB, Gideon HP, Winchell CG, Lin PL, Bingle CD, Snapper SB, Kropski JA, Tyler FJ, Schiller HB, Zaragosi LE, Barbery P, Leslie A, Kiem HP, Flynn JL, Fortune SM, Berger B, Finletter RW, Kean LS, Garber M, Schmidt AG, Lingwood D, Shalek AK, Or dovas-Montanes J, Banovich N, Barbery P, A, Desai T, Duong TE, Eckelberg O, Fark C, Faranc M, Manz G, Hafner A, Horvath P, Huang N, Kaminiki A, Krasnow M, Kropski JA, Kuhnen-mund M, Layfayis R, Lee H, Leroy S, Linnarson N, Lundeberg J, Mayer K, Misharin A, Nawijn M, Nikolik MZ, Or dovas-Montanes J, Pe’er D, Powell J, Quake S, Rajagopal J, Tata PR, Rawlins EL, Regev A, Reyma PA, Rojas M, Rosen O, Saeb-Parsy K, Samakovlis C, Schiller H, Schultz NL, Seibold MA, Shalek AK, Shepherd D, Spence J, Spira A, Sun X, Teichmann S, Theis F, Tsankov A, van den Berge W, Vukovic M, Talaieder F, Mead BE, Guo Z, Wang JP, Gras D, Plaisant M, Ansari M, Angelides I, Adler H, Sucre JMS, Taylor CJ, Lin B, Wahagry A, Mitsiatis S, Dwyer DF, Buschle KM, Boyce JA, Barrett NA, Laidlaw TM, Carroll SL, Colonna L, Tkachev V, Peterson CW, Yu A, Zheng HB, Gideon HP, Winchell CG, Lin PL, Bingle CD, Snapper SB, Kropski JA, Tyler FJ, Schiller HB, Zaragosi LE, Barbery P, Leslie A, Kiem HP, Flynn JL, Fortune SM, Berger B, Finletter RW, Kean LS, Garber M, Schmidt AG, Lingwood D, Shalek AK, Or dovas-Montanes J, Banovich N, Barbery P, A, Desai T, Duong TE, Eckelberg O, Fark C, Faranc M, Manz G, Hafner A, Horvath P, Huang N, Kaminiki A, Krasnow M, Kropski JA, Kuhnen-mund M, Layfayis R, Lee H, Leroy S, Linnarson N, Lundeberg J, Mayer K, Misharin A, Nawijn M, Nikolik MZ, Or dovas-Montanes J, Pe’er D, Powell J, Quake S, Rajagopal J, Tata PR, Rawlins EL, Regev A, Reyma PA, Rojas M, Rosen O, Saeb-Parsy K, Samakovlis C, Schiller H, Schultz NL, Seibold MA, Shalek AK, Shepherd D, Spence J, Spira A, Sun X, Teichmann S, Theis F, Tsankov A, van den Berge M, von Papen M, Whitsel J, Xavier R, Xu Y, Zaragosi LE, Zhang K. HCA Lung Biological Network. SARS-CoV-2 receptor ACE2 is an interferon-stimulated gene in human airway epithelial cells and is detected in specific cell subsets across tissues. Cell 181: 1016–1035.e19, 2020. doi: 10.1016/j.cell.2020.04.035.