Mechanical ventilation stimulates expression of ACE2, the receptor for SARS-Cov-2

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The SARS-Cov-2 virus, which causes COVID 19, uses the cell surface protein ACE2 as receptor for entry into cells. Critically ill COVID-19 patients often require prolonged mechanical ventilation which can cause mechanical stress to lung tissue. In vitro studies have shown that expression of ACE2 in alveolar cells is increased following mechanical stretch and inflammation. Therefore, we analyzed transcriptome datasets of 480 (non-COVID-19) lung tissues in the GTex tissue gene expression database. We found that mechanical ventilation of the tissue donors increased the expression of ACE2 by more than two-fold (p<10^−6). Analysis of transcriptomes of mechanically ventilated mice deposited in the GEO database indicates that this alveolar cell response to stretch and inflammation is mediated by the chemokine midkine. We also found in transcriptomes of the LINCS database of pharmacological perturbations that corticosteroids down-regulate midkine in pulmonal cells, consistent with transcriptome data of animal studies in GEO. Thus, mechanical ventilation of patients with COVID-19 pneumonia may eo ipso facilitate viral propagation in the lung, further accelerating the pulmonal pathology that has necessitated mechanical ventilation in the first place. This vicious cycle offers a possible rationale for interventions that disrupt the corticosteroid-midkine-ACE2 axis and provides a mechanism that supports the calls for gentler ventilation protocols.

A large proportion of COVID-19 patients requires invasive mechanical ventilation over a prolonged period and at higher pressure than in other viral pneumonia1,2. The observation that some patients suddenly deteriorate under mechanical ventilation suggests a “critical transition” (or “tipping point”, in the mathematical sense)3 which often is caused by an underlying positive feedback loop. It has been suggested that mechanical ventilation, while clinically necessary, may contribute to lung injury, and therefore, departure from standard high positive end-expiratory pressure (PEEP) protocols4 and even first-line ECMO have been proposed5 for treatment of COVID-19 patients.

Here we propose that the proclivity of COVID-19 patients to suddenly exacerbate when mechanically ventilated is linked to the fact that SARS-Cov-2, the virus that causes COVID-19, depends on the ACE2 cell surface protein as a receptor for entry into cells6 (Fig. 1). In the airways, ACE2 is expressed mostly in the nasopharyngeal epithelium and minimally in the lung, where it can be found in a small fraction of the type 2 alveolar cells (unpublished and refs. 7,8), the cells that have been implicated in protection against ARDS9. ACE2 expression is elevated in the cardio-pulmonal system in several pathological conditions associated
with an unfavorable course of COVID-19, such as arterial and pulmonal hypertension, chronic heart failure and diabetes\textsuperscript{10,11}. But most importantly, in human lung epithelial cell cultures, ACE2 expression has been observed to be upregulated by mechanical stress (stretching of cells), notably in conjunction with inflammation\textsuperscript{12}. This would establish a “perfect storm” constellation for spread of the SARS-Cov-2 virus in lungs that are infected, inflamed and mechanically ventilated, thereby establishing a positive feedback loop that erects a critical transition ("tipping") point\textsuperscript{3} at which ventilated patients either recover or suddenly deteriorate. Here we analyzed large tissue transcriptome databases and found that ACE2 transcripts was indeed increased individuals on mechanical ventilator.

RESULTS

Data and Literature Analysis: Up-Regulation of Ace2

To determine whether mechanical ventilation enhances ACE2 expression in vivo, we first took advantage of the public GTEx database of human tissue gene expression profiles (https://www.gtexportal\textsuperscript{13}). In a dataset encompassing more than 480 lung transcriptomes, our analysis revealed that mechanical ventilation of tissue donors before death resulted in a statistically significant increase of ACE2 transcript abundance in the lung by more than 2-fold ($p<10^{-6}$; Fig. 2A). This is consistent with cell culture studies in which lung epithelial cells exposed to mechanical stretch and chemical injury (low pH) displayed sustained elevation of ACE2 mRNA expression by 2-4 fold within two days\textsuperscript{12}. Furthermore, transcriptomes deposited in the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo) revealed that in mice, mechanical ventilation together with inflammation (triggered by inhalation of LPS) increased ACE2 expression in the lung by more than 2-fold (GEO, GSE18341)\textsuperscript{14}. (Interestingly, although not further analyzed in detail here, the GTEx transcriptomics data, as shown in Fig. 2A, also suggest that ACE2 mRNA expression in the lung increases with age.)

The upregulation of ACE2 transcripts in lung epithelial cells by mechanical stretch might be mediated by the chemokine midkine (encoded by the MDK gene) because mice deficient of the MDK gene were less susceptible to ARDS and to ARDS-associated fibrosis in an HCl-aspiration/PEEP animal model\textsuperscript{12}. Indeed, our transcriptome analysis in a GEO data set (Fig. 2B)\textsuperscript{13} also revealed a mild but significant increase of MDK mRNA in mice receiving mechanical ventilation (GEO, GSE18341).

The Role of Corticosteroids

Glucocorticoids are used in the treatment of ARDS because of the central role of inflammation in its pathogenesis. However, their use in COVID-19 remains debated and is currently not recommended\textsuperscript{15}. To determine if corticosteroids affect midkine synthesis in human lung tissue, we used SPOKE\textsuperscript{16}, a meta-database containing a vast biomedical knowledge network. Such knowledge networks represent a burgeoning genre of bioinformatics tools that allow investigators to “connect the dots” of elementary biological facts across disparate domains of biomedical knowledge\textsuperscript{17}. SPOKE network analysis revealed that dexamethasone inhibited the expression of the MDK gene in a lung cell line. This finding was extracted from transcriptomes of a systematic perturbation of cell lines by pharmacological compounds (LINCS, http://www.lincsproject.org/)\textsuperscript{18}. This unpublished finding, hidden in the rapidly accumulating omics-databases on
the web, but exposed by SPOKE, was consistent with published transcriptomes of human type 2 alveolar cells in culture treated with dexamethasone (along with differentiation-maintaining factors). Our analysis of these transcriptomes (GEO, GSE 19699) revealed that dexamethasone significantly reduced the expression of midkine mRNA (Fig. 2C).

Administration of corticosteroids in ARDS associated with SARS and MERS has not been shown to improve outcome, and thus, corticosteroids are not recommended in the management of COVID-19. However, in a small retrospective study, administration of methylprednisolone appeared to increase survival of COVID-19 patients with ARDS (HR=0.38, CI=0.20-0.72). There exist also anecdotal reports of benefit of high-dose inhaled corticosteroid (cicleonide) from Japan.

In COVID-19, corticosteroid treatment may in principle disrupt the self-propelling positive-feedback loop of viral propagation and deterioration of lung function which triggers and is triggered by mechanical ventilation of virus-infected lungs. Corticosteroids may mitigate the course of ARDS not only through their anti-inflammatory action, but also by suppression of midkine-mediated induction of the viral receptor ACE2 on type 2 alveolar cells.

While ACE2 is the port for viral entry to the cell, it has a putative protective function in lung injury, implying that lowering ACE2 levels with corticosteroids may be cutting with a double-edged sword. Conversely, ACE2 upregulation by mechanical injury may constitute a natural protective response. Moreover, corticosteroids may promote viral spread because of its immunosuppressive activity. However, along with the anti-inflammatory and cell physiological action of steroids, it may be that in this specific dilemma, the beneficial edge of ACE2 suppression in stressed and inflamed alveolar cells by corticosteroid dominates.

**DISCUSSION**

Mechanical stress of alveolar tissue in combination with inflammation are pivotal factors in the development of ARDS and ensuing fibrosis. The finding from our data analysis that expression of ACE2 in lung cells is increased by mechanical stress and the fact that the SARS-Cov-2 virus uses ACE2 to infect cells, jointly suggest a fateful constellation of a vicious cycle that may explain a dynamics with a tipping point at which COVID-19 pneumonia patients placed on mechanical ventilation would either recover or enter a precipitous course. Supporting evidence for the proposed mechanism could be obtained by the following clinico-pathological measurements:

1. Determination of ACE2-expression of type 2 pneumocytes (and capillary endothelial cells) in postmortem lung tissue specimen of COVID-19, ventilated versus not-mechanically ventilated patients, including those that have received ECMO (extracorporeal membrane oxygenation).

2. Measurement of circulating midkine levels in patients in the clinic before and after they have been placed on mechanical ventilation, and compare patients who have received corticosteroids to those that have not. If midkine in circulation can be shown to be elevated in COVID-19 patients at risk for respiratory failure, it may also serve as a specific biomarker for indication of steroid therapy.

The vicious cycle between pulmonal expression of the viral receptor ACE2 and mechanical ventilation could be disrupted by adjustments of clinical practice that have already been proposed:
More conservative indication for mechanical ventilation or using gentler protocols, as already advocated by some clinicians\(^4\),\(^27\), and even the relaxation of criteria for ECMO \(^4\) to spare the lung from mechanical injury.

Consideration of use of short-term methylprednisolone to COVID-19 patients with ARDS. Indeed, an inflammatory biomarker-guided dosing (CRP) use of methylprednisolone in critically ill pneumonia patients (independent of COVID-19) is in clinical trial (https://clinicaltrials.gov/ct2/show/NCT03852537).

Because of intertwined feedback control loops in the human body, many medical interventions are double-edged swords – as most prosaically manifest by here by the potential “backfiring” of too aggressive mechanical ventilation or by corticosteroid treatment meant to avert an acute threat. A way to safely wield double-edge words to achieve net therapeutic benefit is to employ a dynamically adaptive, minimalist intervention that is guided by close monitoring of markers, including here not only oxygenation but also circulating inflammatory markers, including, possibly midkine levels.

**Contributors**

S.H. formulated the hypothesis and drafted the paper; A.K. led the literature search and microarray data analysis and co-wrote the manuscript; M.S. performed the GTex analysis; B.S. helped with the SPOKE bioinformatics analysis.

**Declaration of interests**

The authors declare no competitive interest.

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Figure 1. Proposed pathways for how mechanical stress from invasive ventilation triggers expression of ACE2, the receptor for viral entry, starting a cascade that promotes viral propagation in the lung of COVID-19 patients, and for intervention opportunities to disrupt a vicious cycle.

Figure 2. Expression of ACE2 and MDK mRNAs in transcriptomes. **A.** Transcriptome (RNAseq) data extracted from GTEx databases showing the increase in ACE2 mRNA in TPM units. Number N of donor per age group and p-value (Whitney-Mann test) are as follows: age 40-49: N=93; p=4.18x10^-7; age 50-59: N=200, p=5.98x10^-9; age 60-69: N=190, p=1.61x10^-6. **B.** mRNA expression of MDK from GEO (GSE 18341) microarray profiles for N=5 mice (each bar = 1 animal) treated with (or without) lipopolysaccharide (LPS) inhalation as indicated prior to 2h of mechanical ventilation. *p=0.014. **C.** mRNA expression of MDK from GEO (GSE 19699) microarray profiles of human type II alveolar cells treated in culture (5 replicates) with dexamethasone and the differentiation factors cAMP and IBX for 5 days. *p=0.016.