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Mechanical ventilation stimulates expression of the SARS-Cov-2 receptor ACE2 in the lung and may trigger a vicious cycle

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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 ($p<10^{-6}$). Analyses of transcriptomes of mechanically ventilated mice in the GEO database indicate that this alveolar cell response to stretch and inflammation is mediated by the chemokine midkine. Using a novel big knowledge network approach (SPOKE) we also found in transcriptomes of pharmaco-logical perturbations (LINCS) that corticosteroids down-regulate midkine in pulmonal cells, and con-firmed this in GEO transcriptomes of animal studies. Thus, mechanical ventilation of patients with COVID-19 pneumonia may *eo ipso* facilitate viral propagation in the lung, further accelerating the pul-monal pathology that has necessitated mechanical ventilation in the first place. This vicious cycle pre-sents a rationale for the temporary treatment with corticosteroids to modulate the midkine-ACE2 axis in ventilated COVID19 patients and 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 pneumonia[1,2]. 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) protocols[4] and even first-line ECMO have been proposed[5] 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 cells[6] (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 ARDS[9]. 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[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[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[3] at which ventilated patients either recover or suddenly deteriorate.

In this big data bioinformatics approach we analyzed various tissue transcriptome databases and found that ACE2 transcripts was indeed increased in lung tissues of individuals that have been on mechanical ventilator. Using a novel big knowledge network approach, we also discovered in pharmacological perturbation transcriptomes that corticosteroids, via down-regulation of midkine, may mitigate stress-stimulated expression of ACE2, which thus, suggest a potential benefit of corticosteroids specifically in COVID19 patients placed on mechanical ventilation.

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)[13]. Analysis of a dataset encompassing more than 480 lung transcriptomes 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 was 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[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)[14]. Interestingly, although not further analyzed in detail here, the GTEx transcriptome data, as shown in Fig. 2A, also suggest that ACE2 mRNA expression in 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[12]. Indeed, our transcriptome analysis in a GEO data set (Fig. 2B)[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[15]. To determine if corticosteroids affect midkine synthesis in human lung tissue, we used SPOKE[16], a meta-data-

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base containing a vast biomedical knowledge network that seamlessly transcends various biomedical disciplines. Such "big knowledge graphs" represent a burgeoning genre of bioinformatics tools that allow investigators to connect the dots of biological facts across disparate domains of biomedical knowledge[17]. SPOKE network anlaysis 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/)[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)[19]. 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 currently not recommended in the management of COVID-19 [15,20]. 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) [21]. There is also anecdotal reports of benefit of high-dose inhaled corticosteroid (cicleconide) from Japan [22].

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 specifically in COVID19 patients not only through their anti-inflammatory action, but also by suppression of midkine-mediated induction of expression of the SARS-Cov-2 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 [23], 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 reponse. Moreover, corticosteroids may promote viral spread because of its immunosuppressive activity, further enhancing the double-edge sword effect. 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[12,14,24,25]. 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[26] 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:

(1) 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.

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

[NOTE ADDED in v2:] Early results released from the RECOVERY study of COVID-19 treatments suggest that dexamethasone reduces death by one third in pateints on mechanical ventilation, more so than in patients that only receive oxygen, while there was no benefit for patients who did not require respiratory support. These findings, notably the differential benefit for pateints on mechanical ventilation, is consistent with the proposed mechanism that corticosteroids not only non-specifically reduce inflammation but also may suppresses injury-induced expression of ACE2 in the lung that allows the SARS-Cov-2 virus to propagate.

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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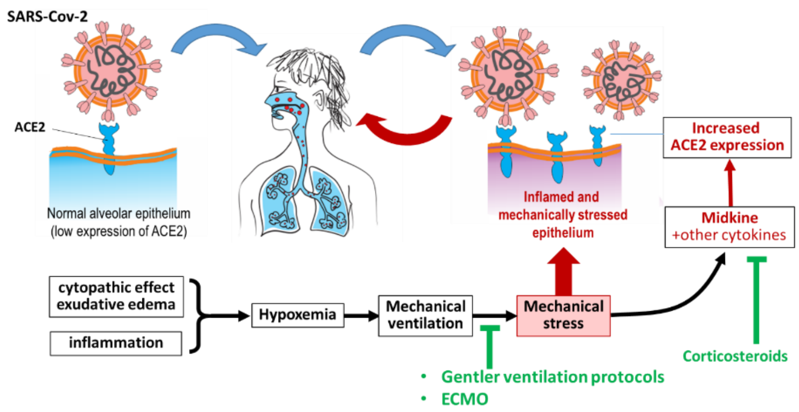

Figure 1. Proposed pathways for how mechanical stress from invasive ventilation triggers expression of ACE2, the receptor for viral entry, starting a cascade that promotes viral propagation in the lung of COVID-19 patients, and for intervention opportunities to disrupt a vicious cycle.

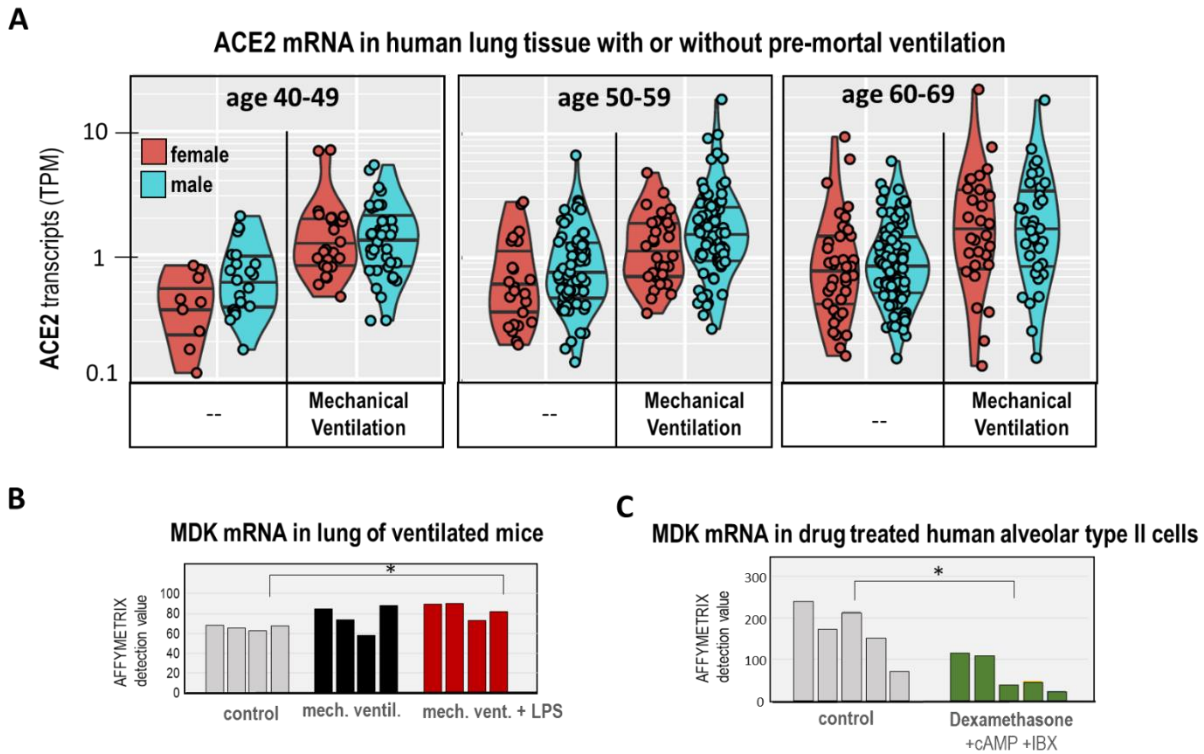

Figure 2. Expression of ACE2 and MDK mRNAs in transcriptomes. ***A.*** Transcriptome (RNAseq) data extracted from GTEx databases showing the increase in ACE2 mRNA in TPM units. Number N of donor per age group and *p*-value (Whitney-Mann test) are as follows: age 40-49: N=93; $p=4{\cdot}18\times10^{-7}$; age 50-59: $N=200$, $p=5{\cdot}98\times10^{-9}$; age 60-69: $N=190$, $p=1{\cdot}61\times10^{-6}$ ***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. *$p=0{\cdot}014$. **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. *$p=0{\cdot}016$.