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Short Communication

Stroke increases the expression of ACE2, the SARS-CoV-2 binding receptor, in murine lungs

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ABSTRACT

Background: The newly emerged severe acute respiratory syndrome coronavirus (SARS-CoV-2) has caused a worldwide pandemic of human respiratory disease. Angiotensin-converting enzyme (ACE) 2 is the key receptor on lung epithelial cells to facilitate initial binding and infection of SARS-CoV-2. The binding to ACE2 is mediated via the spike glycoprotein present on the viral surface. Recent clinical data have demonstrated that patients with previous episodes of brain injuries are a high-risk group for SARS-CoV-2 infection. An explanation for this finding is currently lacking. Sterile tissue injuries including stroke induce the release of several inflammatory mediators that might modulate the expression levels of signaling proteins in distant organs. Whether systemic inflammation following brain injury can specifically modulate ACE2 expression in different vital tissues has not been investigated.

Methods: For the induction of brain stroke, mice were subjected to a surgical procedure for transient interruption of blood flow in the middle cerebral artery for 45 min and sacrificed after 1 and 3 days for analysis of brain, lung, heart, and kidney tissues. Gene expression and protein levels of ACE2, ACE, IL-6 and IL1β were measured by quantitative PCR and Western blot, respectively. The level of soluble ACE2 in plasma and bronchial alveolar lavage (BAL) was measured using an immunoassay. Immune cell populations in lymphoid organs were analyzed by flow cytometry. Post-stroke pneumonia in mice was examined by bacterial cultures from lung homogenates and whole blood.

Results: Strikingly, 1 day after surgery, we observed a substantial increase in the protein levels of ACE2 in the lungs of stroke mice compared to sham-operated mice. However, the protein levels of ACE2 were found unchanged in the heart, kidney, and brain of these animals. In addition, we found increased transcriptional levels of alveolar ACE2 after stroke. The increased expression of ACE2 was significantly associated with the severity of behavioral deficits after stroke. The higher protein levels of alveolar ACE2 persisted until 3 days of stroke. Interestingly, we found reduced levels of soluble ACE2 in plasma but not in BAL in stroke-operated mice compared to sham mice. Furthermore, stroke-induced parenchymal and systemic inflammation was evident with the increased expression of IL-6 and IL-1β. Reduced numbers of T-lymphocytes were present in the blood and spleen as an indicator of sterile tissue injury-induced immunosuppression.

Conclusions: We demonstrate specific augmented alveolar ACE2 levels and inflammation in murine lungs after experimental stroke. These pre-clinical findings suggest that patients with brain injuries may have increased binding affinity to SARS-CoV-2 in their lungs which might explain why stroke is a risk factor for higher susceptibility to develop COVID-19.

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1. Introduction

Angiotensin-converting enzyme (ACE) 2 is present in mammalian tissues and plays an important role in the resolution of inflammation and cellular homeostasis under inflammatory conditions. The stimulation of ACE2 with specific activators is protective in specific diseases, such as brain injury induced by an ischemic stroke (Bennion et al., 2015; Mecca et al., 2011). However, recent data have demonstrated that SARS-CoV-2, the coronavirus causing COVID-19, utilizes ACE2 for entering into the epithelial cells (Hoffmann et al., 2020). The ensuing infection is accompanied by inflammatory lung injury and death and has caused a worldwide epidemic since its start at the end of 2019 (Pedersen and Ho, 2020). Although the lungs are the major target of the virus, its spread to the heart, kidney, and brain has also been observed in human patients (Trypsteen et al., 2020). This multi-organ target of the virus infection has been associated with the dysfunction of affected organs and poor survival (Chen et al., 2020).

Stroke-induced immune activation can affect multiple vital organs and augment the progression of specific co-existing inflammatory diseases. Previous findings have shown that the induction of stroke in murine models of brain ischemia can activate immune cells and promote the progression of inflammatory heart disease (Roth et al., 2018). Moreover, brain injury can modulate the functions of the intestine and its immune components, supporting the hypothesis of multi-organ failure after stroke (Singh et al., 2016a, 2018). In addition, stroke patients may present signs of severe immunosuppression and inflammation that often lead to hospital-acquired respiratory infections (Shi et al., 2018). A recent study by Austin et al. has demonstrated an increased number of mononuclear granulocytes in bronchoalveolar lavage fluid (BAL) and higher IL-1β expression in lung tissue of mice that were subjected to ischemia-induced brain injury (Austin et al., 2019). In this respect, it is highly conspicuous that recent studies have suggested that patients with cardiovascular diseases and stroke form a high-risk group for SARS-CoV-2 infection (Bravi et al., 2020; Fifi and Mocco, 2020; Nishiga et al., 2020; Ssentongo et al., 2020). Besides, there are a plethora of clinical studies that identified severe episodes of stroke in COVID-19 patients (Fatima et al., 2020; Katz et al., 2020; Kihira et al., 2020). However, whether post-stroke immune alterations and lung inflammation might increase the susceptibility of patients to SARS-CoV-2 infection is currently enigmatic and requires careful examination.

Hence, we hypothesized that brain injury-induced immunological alterations and systemic inflammatory conditions may modulate the expression of ACE2 in different vital organs and thereby, promote binding and infection of SARS-CoV-2. At present, there is no data available demonstrating the dynamics of membrane-bound and soluble ACE2 in murine tissues after sterile brain injury. Here, using an experimental murine model of stroke we found that focal cerebral ischemia specifically increased the expression of ACE2 in the lungs but not in other vital organs.

2. Results

To study the impact of stroke on the dynamics of ACE/ACE2 in different vital tissues, we used a transient stroke-reperfusion mouse model which induces large focal brain lesions and severe neurological deficits (Suppl. Fig. 1A–C). Interestingly, Western blot analysis of mouse lungs showed a 2–fold increase in ACE2 protein abundance compared to the sham controls one day after post-ischemic-reperfusion injury.

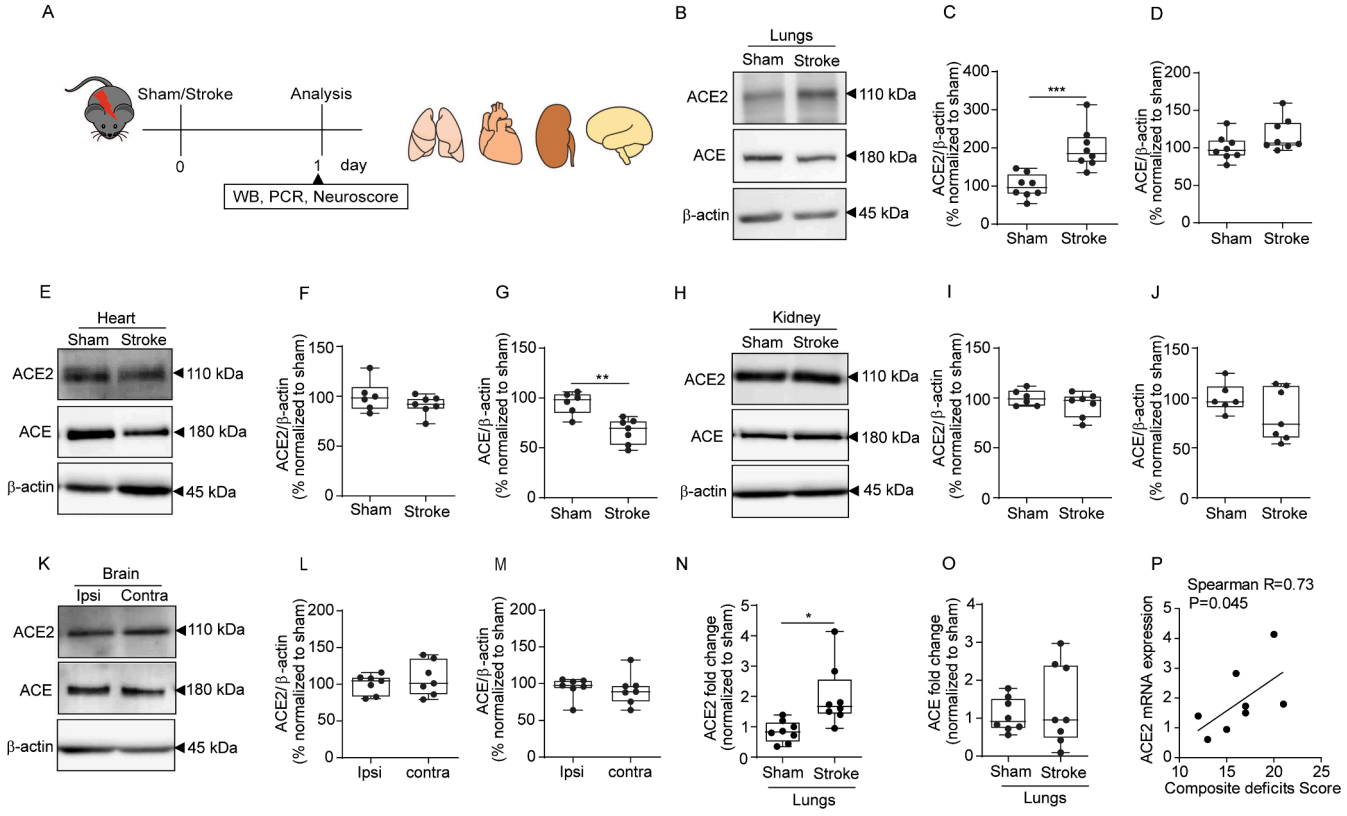

Fig. 1. Stroke increases ACE2 protein levels and gene expression in the lung. A. Graphical representation of the experimental protocol with timeline. B, E, H, K. Representative Western blots for lung, heart, kidney and brain ACE2, ACE, and β-actin protein in sham and stroke-operated mice after 1 day. C, D) Increased levels of ACE2 but not ACE in the mice lung after 1 day of stroke. F, G) No change in ACE2 but decreased levels of ACE in the mice heart after stroke. I, J) No change in the levels of ACE2 and ACE in the mice kidney. L, M) No change in the levels of ACE2 and ACE in mouse brains after 1 day of stroke. N, O) Increased gene expression of ACE2 but not ACE in mouse lungs 1 day after stroke. P) A positive correlation between composite deficits scores and lung ACE2 expression in stroke mice. ***p < 0.001, **p < 0.01, *p<0.05, Mann-Whitney *U* test, N = 7–8 per group.

However, ACE protein levels remained unchanged (Fig. 1B–D). We further investigated the levels of ACE2 and ACE in heart, kidney and brain tissue after stroke- or sham-surgery but only found reductions in ACE protein levels in heart of stroke mice compared to sham-operated mice (Fig. 1E–M). These results indicate a specific effect of stroke on the expression of ACE2 in murine lungs. We further sought to determine if ACE2 expression in murine lungs was regulated at the transcriptional level and performed qPCR analysis on lung tissues 1 day after stroke- or sham-surgery. Strikingly, the induction of stroke significantly increased the level of ACE2 mRNA in the lungs compared to sham-operated mice (Fig. 1N). Again, the expression of ACE mRNA was not altered between sham and stroke mice (Fig. 1O). Interestingly, stroke-induced severe neurological deficits in mice were positively correlated with the increased expression of alveolar ACE2 (Fig. 1P).

Furthermore, we found an increased expression of the proinflammatory cytokines IL-6 and IL-1β mRNAs in murine lungs after stroke (Fig. 2B, C). These data suggest that stroke-induced peripheral inflammation might increase ACE2, but not ACE levels, and generate an inflammatory milieu in the lungs of affected mice. Interestingly, our results also showed increased gene expression of the pro-inflammatory cytokines IL-6 and IL-1β in the ipsilateral compared to contralateral brain hemispheres in stroke mice (Fig. 2D, E).

Further, to investigate if the induction of stroke changes the levels of soluble ACE2, we measured the ACE2 concentrations in plasma and BAL using an immunoassay. Strikingly, we found reduced levels of ACE2 in plasma but not in BAL of stroke-operated mice compared to the sham controls after 1 day (Fig. 2F, G). In addition, mice subjected to stroke had a reduced number of circulating and splenic T lymphocytes which is an indicator of stroke-induced systemic immunosuppression (Fig. 2H, I). Post-stroke pneumonia is one of the comorbidities that can induce lung inflammation and change the expression of different signaling proteins. To verify this, we analyzed the presence of pneumonia-associated symptoms in mice using bacterial cultures and physiological parameters. We found that the induction of large focal brain lesions in mice reduced their body weight and temperature but did not show positive bacterial growth from lung and blood tissues on day 1 (Suppl. Fig. 2A–E).

Furthermore, we studied the longevity of ACE2 abundance in the lungs of mice after 3 days of stroke. Western blot analysis still showed increased abundance of ACE2 protein but not ACE in murine lungs after stroke compared to sham-operated animals (Fig. 2J–M). However, as opposed to day 1 no reduction in the plasma ACE2 was found after 3 days of stroke (Fig. 2N).

In conclusion, our results showed that sterile brain injury can modulate the levels of ACE2 on alveolar membranes and in the circulation. This may explain the higher susceptibility of stroke patients for COVID-19.

3. Discussion

ACE and ACE2 are the key enzymes of the renin-angiotensin system which regulate blood pressure and salt-fluid balance in the body (Donoghue et al., 2000). ACE2 is a homologue of ACE and counterbalances pathways of inflammation in specific tissue injuries (Rodrigues

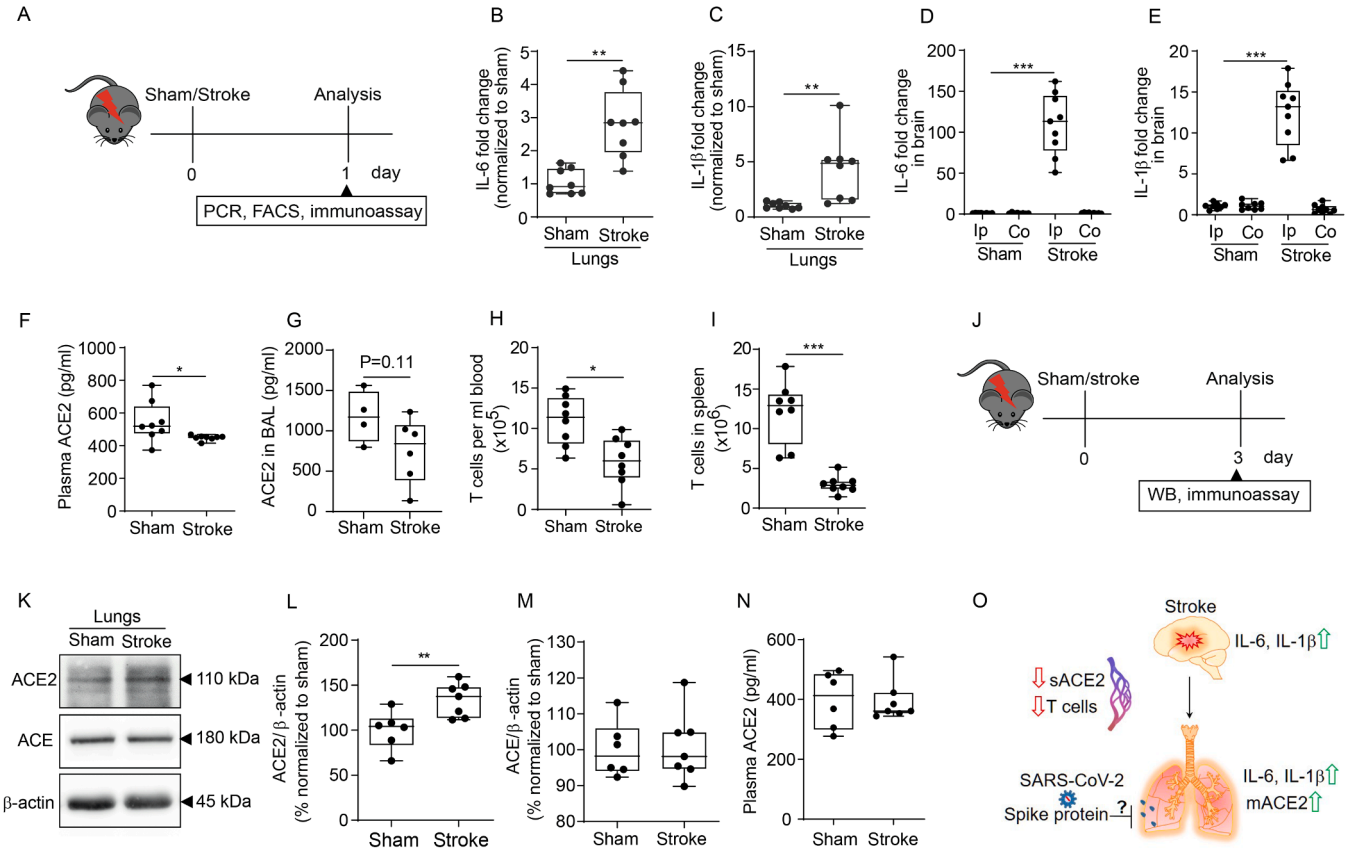

Fig. 2. Stroke induces inflammation and peripheral immunosuppression and reduces soluble ACE2 levels. A. Graphical representation of the experimental protocol with timeline. B, C. Higher gene expression of IL-6 and IL-1β cytokines in the lung of stroke mice. D, E. Increased gene expression of brain IL-6 and IL-1β in ischemic (Ipsi) hemispheres compared to non-ischemic hemispheres (Contra). F, G. Reduced levels of ACE2 in plasma but not BAL after 1 day of stroke compared to sham controls. H, I. Reduced number of T lymphocytes in blood and spleen of stroke mice. J. Graphical representation of the experimental protocol with timeline. K. Representative Western blots for lung ACE2, ACE, and β-actin protein in sham and stroke mice after 3 days. L, M. Increased levels of ACE2 but not ACE in the mice lung after 3 days of stroke. N. No change in the levels of ACE2 in plasma after 3 day of stroke compared to sham controls. O. Graphical abstract demonstrating stroke-induced alterations in systemic immunity and ACE2 levels. ***p < 0.001, **p < 0.01, *p < 0.05, Mann-Whitney *U* test, N = 4–8 per group.

Prestes et al., 2017). A higher expression of ACE2 is protective in lung injury, and the stimulation of this pathway with chemical activators has been shown to reduce LPS-induced lung edema and inflammatory tissue damage (Li et al., 2016). A recent study by Imai et al. suggested that the loss of ACE2 in a mouse model of acid-induced lung injury leads to the worsening of lung inflammation and increased lung edema (Imai et al., 2005).

ACE2 is utilized as a binding receptor by SARS-CoV2 for its entry into nasal and lung epithelial cells and the infection can induce acute respiratory distress syndrome (ARDS) in affected patients (Hoffmann et al., 2020; Ziegler et al., 2020). In addition, higher expression of ACE2 has been found on the lung epithelial cells of COVID19 patients (Chua et al., 2020). The tissues with higher expression of ACE2 are the major targets of SARS-CoV2 that may promote multi-organ failure in patients with COVID19 (Xu et al., 2020). In addition, a lower expression of alveolar ACE2 in young children compared to adults has been associated with a lower prevalence of COVID19 in children (Bunyavanich et al., 2020; Saheb Sharif-Askari et al., 2020). Based on the fact that SARS-CoV2 infection co-exists with reported cases of cardiac disease and stroke patients (Shi et al., 2020; Zhai et al., 2020), we hypothesized that brain injury may also modulate the ACE2 expression in different vital tissues such as lung, heart, kidney, and brain. Indeed, our results suggest a rapid and specific increase of ACE2 expression and pro-inflammatory cytokines in murine lungs after ischemic brain injury. The increase of ACE2 levels within 1 day of brain injury suggests fast kinetics of signaling to induce protein expression changes in the lungs. The increase in alveolar ACE2 protein levels was evident after 3 days of brain injury. Interestingly, our data did not show the increase in ACE2 levels in murine heart, kidney and brain on 1 day after brain tissue injury. Considering the longer incubation time (5–12 days) of SARS-CoV2 infection (Lauer et al., 2020), investigations on alveolar ACE2 alterations in the chronic phases of brain injury will be important for future investigations. The molecular mechanisms underlying this response in the lungs after stroke are currently unclear, but, based on our data and related studies of others increased post-stroke lung inflammation might be a possible explanation (Austin et al., 2019). Physiologically, the augmented ACE2 levels in inflamed lungs after brain ischemia may help to counterbalance the subsequent inflammatory lung injury (Imai et al., 2005). Nevertheless, in the presence of a virus that exploits this anti-inflammatory enzyme for cell entry and infection, this protective mechanism might prove fatal.

One of the clinical features of SARS-CoV-2-infected patients is the ensuing cytokine storm that leads to ARDS (Mehta et al., 2020). In the last months, several clinical studies have shown a higher serum level of cytokines such as IL-1β, IL-6 and TNF-α in virus-infected patients compared to the healthy controls (Conti et al., 2020; Han et al., 2020). Our results show that brain injury can also increase the expression of the pro-inflammatory cytokines IL-1β and IL-6 in the injured brain hemispheres and the lungs. In addition, sterile tissue injury can initiate the release of damage-associated molecular patterns (DAMPs) and thereby propagate parenchymal and systemic inflammation (Singh et al., 2016b). These DAMPs might serve as activation signals for lung epithelial, endothelial, and immune cells. However, which specific molecules modulate ACE2 expression in the lungs after brain injury is an open question and requires further research.

The potential role of soluble ACE2 as a therapeutic target for SARS-CoV-2 infection is intensively discussed in the scientific community (Tang et al., 2020). Recent experimental data have demonstrated the effectiveness of recombinant human ACE2 in blocking SARS-CoV-2 binding to membrane ACE2 and thereby blocking virus invasion (Monteil et al., 2020). The safety and use of soluble ACE2 therapy in humans has been successfully tested in a phase 1 clinical trial (Haschke et al., 2013) and is now examined in a clinical trial to treat COVID-19 patients (Clinicaltrials.gov #NCT04335136). In this respect, it is possible that decreased concentrations of soluble ACE2 in serum, as seen in our experimental animals and as shown in stroke patients (Bennion et al., 2016) together with increased levels of alveolar ACE2 after stroke may promote virus binding to lung epithelia. Interestingly, our experiments also showed reduced levels of plasma ACE2 after 1 day of stroke compared to sham surgery. Recently, a study by Bennion et al. demonstrated the increase in the activity of plasma ACE2 after brain ischemia in a rat model of experimental stroke (Bennion et al., 2015). These differences to our results might be due to the utilization of different animal models (mice vs rat) and measurement methods for ACE2 analysis (amount vs activity). Moreover, post-stroke immunosuppression and pneumonia are the key co-morbidity factors contributing to poor outcomes and increased mortality in affected individuals (Kalra et al., 2015). The mouse model of stroke used in our study also indicated a pronounced post-stroke lymphopenia similar to what is commonly observed after human stroke. However, in our study the symptoms of pneumonia were absent in the used mouse model of stroke. Collectively, we suggest that increased ACE2 levels in lungs, systemic immunosuppression and reduced circulating ACE2-levels may act together to increase the prevalence of severe courses of COVID-19 in patients with preexisting brain injuries.

In conclusion, our results demonstrate that in the lungs of brain-injured mice ACE2 is rapidly upregulated and accompanied by increased inflammatory responses. The results described in this study may help to understand the possible mechanisms behind the known increased susceptibility of brain-injury patients to lung infections, especially in the cases with simultaneous brain tissue injury and COVID-19.

4. Funding and conflict of interest

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2021.01.039.

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