A systematic review of the literature on the relationship between ACE2 and SARS-CoV infection in animal models

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Abstract

Background: similar to the severe acute respiratory syndrome coronavirus (SARS-CoV), research suggests severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) interacts with angiotensin-converting enzyme 2 (ACE2) to gain entry into cells. The primary objective is to synthesize existing animal model research on the association between ACE2 and severe acute respiratory syndrome (SARS) infection. The secondary objective is to describe the consequences of infection on ACE2 expression.

Methods: we performed a systematic literature search of Medline, Embase, and Global Health databases. We included animal studies on the connection between SARS-CoV infection and variations in ACE2 receptor or expression thereof. Included studies were assessed for quality using the CAMARADES checklist.

Results: we included nine studies, all determining the role of ACE2 in SARS-CoV infections. Five low to moderate quality studies showed that increased ACE2 expression was correlated with increased SARS-CoV infection. Five low to moderate quality studies showed post-infection downregulation of ACE2 to be associated with increased clinical symptoms, morbidity, and mortality.

Conclusion: this review shows that pre-infection, greater ACE2 expression correlates with increased infection leading to worse clinical outcomes. Assuming similar mechanisms for SARS-CoV-2 as in SARS-CoV, it is plausible that ACE2 has some role and impact in COVID-19 infections. Further high-quality animal model research is needed to determine the role of ACE2, specifically in COVID-19 infections.

Introduction

In December 2019, a novel coronavirus, since named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was detected as the cause of acute respiratory illnesses in several patients from Wuhan, China.[1] In the span of three months, the resulting disease, coronavirus disease 2019 (COVID-19), had spread to over 150 countries, resulting in over 190 000 cases and causing over 7800 deaths.[1] On March 11, 2020, the World Health Organization (WHO) declared a COVID-19 pandemic.[1]

The use of angiotensin-converting enzyme inhibitors (ACEis) and angiotensin II receptor blockers (ARBs) has been widely debated in the recent coronavirus pandemic. A landmark study by Zhou et al., which identified and characterized the virus, confirmed SARS-CoV-2's use of the angiotensin-converting enzyme II (ACE2) receptor for host cell entry, similar to the severe acute respiratory syndrome coronavirus (SARS-CoV) 18 years ago.[2] SARS-Cov-2 also appears to have greater binding affinity owing to a single mutation in the receptor binding domain.[3] In conjunction, several studies in China showed that severe disease was prominent in hypertension, diabetes mellitus, and coronary artery disease.[4] In addition, over two-thirds of patients who have died from COVID-19 had diabetes or cardiovascular disease, both of which are frequently treated with ACEis and ARBs.[5]

As such, a recent correspondence in Lancet Respiratory Medicine postulates that ACE2 expression is increased with ACEis and ARBs, thus possibly facilitating COVID-19 infection.[6] This has garnered significant attention worldwide with regards to whether

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or not patients should continue using these medications.[7,8] The consequences may be widespread, given that as of 2008 there were 162.8 million and 82.5 million prescriptions in the USA annually for ACEis and ARBs respectively.[9] This number is likely higher now given the rising rates of coronary artery disease as well as this class's use in heart failure, chronic kidney disease, and diabetic nephropathy.

In response, many professional societies including the Canadian Cardiovascular Society, American Heart Association, American College of Physicians, and European Society of Cardiology have released professional statements on this issue.[10] Each of these statements recommends continuing ACEis and ARBs due to lack of convincing evidence otherwise.

In order to address this controversy, a fundamental understanding of ACE2, its function, and its role in the pathogenesis of coronaviruses is warranted. This has been studied in the past for SARS-CoV. ACE2 is highly expressed in the lung alveolar epithelial cells and macrophages. In addition, its expression has been reported in other organs and organ systems including the heart, gut, and kidney.11 It is a potent enzyme known to convert the vasoconstrictor angiotensin II to angiotensin-(1-7). Angiotensin-(1-7) exerts a cardioprotective role, including increased vasodilation, decreased hypertrophy, and decreased thrombosis.[12]

In terms of pathogenesis, both SARS-CoV's and SARS-CoV-2's entry into target cells is a multi-step process beginning with viral particles binding to ACE2 receptors on the cell surface. Following this, TMPRSS2, an essential serine protease in the human cell, is required for spike glycoprotein priming.[13] Thus, ACE2 is not the only factor involved in coronavirus entry. In addition, the membrane bound ACE2 is processed by the metalloproteinase ADAM17 which cleaves the ACE2 ectodomain, releasing it in its soluble form.[14] This soluble form may serve as a decoy receptor as well, which some speculate could hinder, rather than facilitate, infection. For instance, it has been shown that administration of recombinant ACE2 has beneficial effects following respiratory syncytial virus infection in mice.[15] It has also been discovered that recombinant ACE2 protein alleviates the symptoms of acute lung injury in wild-type mice as well as in ACE2 knockout mice.[16] However, it is worth noting that the soluble levels of ACE2 in blood at baseline are typically very low in humans.[17]

There are clearly conflicting schools of thought on ACE2 and its role in SARS infection.[18] ACE2 has repeatedly been demonstrated to interact with SARS-CoV receptor binding domains.[19,20] However, it is unclear whether its upregulation would serve to increase infectivity via the membrane receptor or decrease viral load via competitive action of its shed soluble form. Thus, our study serves to address this gap in understanding by reviewing in vivo studies of SARS infectivity in relation to ACE2 expression. Notably, we will not be addressing the effect of ACEis and ARBs directly. Our primary objective is to conduct a systematic review to synthesize existing preclinical research on the association between ACE2 and infection with SARS-CoV as well as the consequences of SARS-CoV infection on subsequent ACE2 expression. We later discuss the implications of our findings for COVID-19.

Methods

The protocol for this systematic review was registered on PROSPERO (CRD42020176691). We performed a systematic search of the Medline, Embase, and Global Health databases to find articles related to the coronavirus and the ACE2 receptor. The search strategy employed was: ("SARS" OR "severe acute respiratory syndrome" OR "SARS-CoV") AND ("angiotensin converting enzyme 2" OR "ACE2" OR "ACE 2" OR "ACE-2" OR "angiotensin converting enzyme II" OR "ACE II" OR "ACE-II"). All studies addressing ACE2 and SARS were included for review up to March 31, 2020. We limited studies to those published in English only. Three independent reviewers screened the titles and abstracts for inclusion and exclusion. Discrepancies were resolved by discussion. Three independent reviewers then reviewed the full text for inclusion and discrepancies were again resolved by discussion. The inclusion and exclusion criteria are provided below.

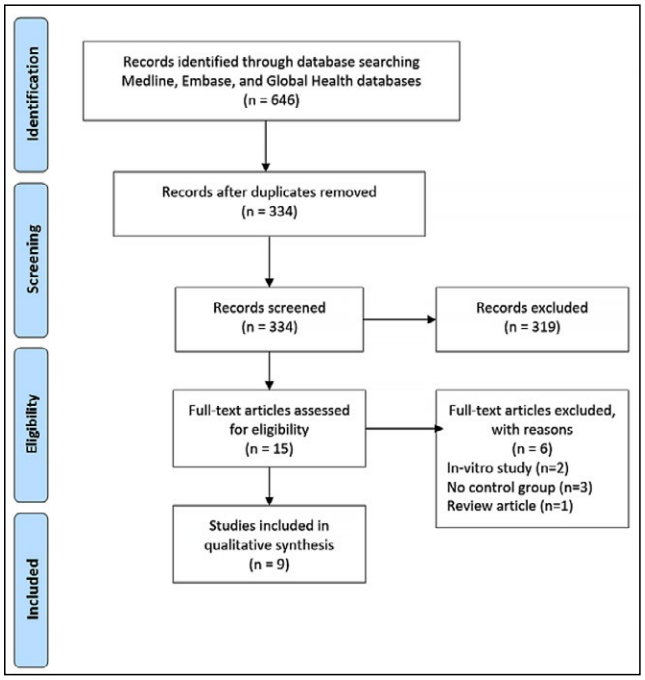

Figure 1. PRISMA flow diagram

Studies were included if they fulfilled the following criteria:

- Animal studies only
- Viruses studied included SARS-CoV
- Independent variables included a variation in ACE2 receptor through variations in receptor gene and/or receptor expression
- Dependent variables included a measure of infection with SARS-CoV

Studies were excluded if they had at least one of the following criteria:

- Non-English language studies
- In-vitro studies
- Review articles
- Inadequate control variables
- Studies looking at genetic phylogeny
- Studies looking at viral entry mechanism

The following data were extracted from each study: title, author, year of publication, independent variables, dependent variables, control variables, virus studied, animal model used, method of measurement of ACE2 expression, method of measurement of SARS infectivity, main outcomes, and conclusions. The data abstraction was done in duplicate with disagreements resolved by the third person.

Data synthesis methods

Experiments related to the infectivity of SARS-CoV and ACE2 expression or activity were included. Experiments aimed at answering similar research questions were identified and grouped together. For each experiment, the conclusions were summarized into a data extraction sheet. Qualitative synthesis of these conclusions was performed.

Results

The search identified 646 articles. After removing duplicates, 334 unique articles were left. Following title and abstract screening, 15 articles were reviewed in full and 9 articles met our final inclusion and exclusion criteria. Our PRISMA flow diagram can be found in Figure 1.

Table 1 shows the animal model, viral strain, and independent and dependent variables in each study. The most commonly used strains were the Beijing strain (PUMC01), used in four studies (19, 21, 24, 28), and the Urbani strain, used in two studies (23, 27). Two studies compared multiple strains of SARS-CoV (22, 26). Six of the nine studies used mice while the remaining three used rats, cats, ferrets, and macquees as the animal models (19, 21-28).

Study quality

Study quality was assessed using the CAMARADES quality checklist and shown in Table 2. Two fields were not applicable to the research question (methods of anesthesia, requirement for the animal model as aged, hypertensive, and diabetic). All studies ranked as low to moderate quality with a total score of 5 or lower.

Table 1. The characteristics of included studies: n = 9

First Author, Year	Animal Model Used	Virus Strain Used	Independent Variable	Dependent Variable
Oudit, 2009 (21)	Mice	SARS-CoV (Beijing strain, PUMC01 isolate)	Infection type: SARS-CoV infection vs. encephalomyocarditis infection vs. normal saline	Cardiac ACE2 expression (RT-PCR) Cardiac ACE2 protein (Western blot)
Rockx, 2009 (22)	Mice: aged vs. young	icUrbani (late, non-lethal human SARS strain), icGZ02 (early lethal human SARS strain), icHC/SZ/61/03 (zoonotic lethal SARS-CoV strain)	Age of mice Virus strain	Clinical findings Peak viral titres ACE2 expression (RT-PCR)
Yoshikawa, 2009 (23)	Mice: AC70 vs. AC22 (two lines transgenic for hACE2)	SARS-CoV (Urbani strain)	Mice type	Clinical findings Histopathology Viral titres SARS-CoV antigen expression (IHC) hACE2 expression (RT-PCR) Cytokine levels (tissue homogenate) Immune cell count
Chen, 2008 (24)	Chinese macaques	SARS-CoV (Beijing strain, PUMC01 isolate)	Rh-ACE2 variations	Histopathology
Van der Brand, 2008 (25)	Cats, ferrets	Strain HKU 39849 of SARS-CoV	Animal type: cats vs. ferrets	Clinical findings Histopathology SARS-CoV antigen expression (RT-PCR) ACE2 expression (RT-PCR)
Nagata, 2007 (26)	Rats: adult vs. young	SARS-CoV (Frankfurt1 isolate and variations thereof)	Age of rats Virus type	Clinical findings Histopathology (lungs) Inflammatory cytokines (serum and lung homogenate) SARS-CoV antigen expression (IHC) ACE2 expression (IHC)
Tseng, 2007 (27)	Mice: transgenic for hACE2 vs. wildtype	SARS-CoV (Urbani strain)	Mice type Virus dose	Clinical findings Viral titres Cytokine levels (organ tissue homogenate) SARS-CoV antigen expression (IHC) ACE2 expression (IHC)
Yang, 2007 (28)	Mice: transgenic vs. wild-type mice	SARS-CoV (Beijing strain, PUMC01 isolate)	Mice type	Peak viral titres Number of infected animals day 7 p.i
Kuba, 2005 (19)	Mice: ACE2 knockout vs. wild-type	SARS-CoV (Beijing strain, PUMC01 isolate)	Mice type	Viral replication SARS Spike RNA

p.i = post infection, IHC = immunohistochemistry, ACE2 = Angiotensin converting enzyme 2, hACE2 = human ACE2

Table 2. CAMARADES quality checklist

First Author, Year	Peer-Reviewed Publication	Control of Temperature	Random Allocation	Blind Inoculation of Virus	Blind Assessment of Outcome	Sample Size Calculation	Compliance with animal Welfare regulation	Conflict of Interest Statement	Total
Oudit, 2009 (21)	1	0	0	0	0	0	1	1	3
Rockx, 2009 (22)	1	1	0	0	0	0	1	1	4
Yoshikawa, 2009 (23)	1	0	0	0	0	0	0	1	2
Chen, 2008 (24)	1	0	0	0	0	0	1	1	3
Van der Brand, 2008 (25)	1	1	0	0	0	0	1	1	4
Nagata, 2007 (26)	1	0	0	0	0	0	1	1	3
Tseng, 2007 (27)	1	0	0	0	0	0	1	1	3
Yang, 2007 (28)	1	1	0	0	0	0	1	0	3
Kuba, 2005 (19)	1	0	0	0	0	0	1	1	3

Co-localization of ACE2 and SARS-CoV

Three studies examined the similarities in location between ACE2 receptor and SARS-CoV antigen using immunohistochemistry (IHC). Van der Brand et al. noted that airway and alveolar cells expressing ACE2 were the same cells infected with SARS-CoV, and this was consistent in both ferrets and cats.[25] Similarly, Nagata et al. found that the extent and location of virus-infected cells correlated with the extent and location of ACE2 expression, especially in the airways of examined rats.[26] Lastly, Tseng et al. found that human ACE2 (hACE2), in transgenic mice, and SARS-CoV antigen localized to the same regions in the lungs and GI tract, but not in the central nervous system.[27]

ACE2 and SARS-CoV infectivity

Five studies used genetically engineered animals to study the effect of ACE2 on SARS-CoV infectivity. Kuba et al. used ACE2 knockout mice and found that they had significantly lower viral titres recovered from lung tissue, lower levels of SARS spike RNA by reverse transcription polymerase chain reaction (RT-PCR), and less histologic lung alterations compared to wildtype controls.[19] Similarly, Oudit et al. showed that SARS-CoV levels, as measured by RT-PCR for RNA polymerase gene, increased in wildtype mice after infection but decreased in ACE2 mutant mice.[21] Yang et al., Tseng et al., and Yoshikawa et al. all used mice transgenic for hACE2 which is known to have higher affinity for SARS-CoV compared to the mouse ACE2.[23,27,28] Yang et al. found that lungs of transgenic mice had higher viral titres, higher levels of SARS-CoV S protein by immunohistochemistry (IHC), and more severe pathologic changes compared to wildtype mice. Additionally, most (8/9) wildtype mice were also able to clear the virus by postinfectious day 7, compared to only 2/9 transgenic mice.[28] Tseng et al. also found that one lineage of transgenic mice (AC70) showed rapid weight loss and respiratory symptoms before dying within eight days of SARS-CoV infection, whereas their wildtype counterparts showed no clinical signs. Furthermore, transgenic mice had higher viral titres, more frequent SARS-CoV antigen detection by IHC, and higher levels of inflammatory cytokines in their lungs compared to wildtypes.[27] Yoshikawa et al. compared the same lineage of transgenic mice (AC70) to another lineage (AC22) which had lower hACE2 expression by RT-PCR. AC22 mice had mild illness and weight loss after infection but eventually recovered clinically and regained weight, while AC70 mice died within 6 days of infection.[23] Although viral titres, histopathologic changes, and viral antigen detection by IHC were comparable in the lungs of both lineages, AC70 mice had lower levels of inflammatory cytokines and more pronounced suppression of cellular immunity, which may explain their clinical response.[23]

ACE2 downregulation

Five studies examined ACE2 expression after SARS-CoV infection. Kuba et al. found that ACE2 expression in the lungs of mice was significantly decreased 2 days post infection.[19] Oudit et al. also reported downregulation after infection but in the hearts of infected mice.[21] Chen et al. found that ACE2 downregulation in Chinese macquee lungs was more pronounced in clinically severe infections, while Rockx et al. found that only lethal strains of SARS-CoV resulted in downregulation of ACE2 in the mouse lungs.[22,24] The latter also found that young mice were resistant to downregulation of ACE2 by one of the lethal strains used. This is consistent with Nagata et al.'s finding that ACE2 was downregulated only in airways of young mice, but also in lung parenchyma of older mice, indicating that the ACE2 downregulation effect by SARS-CoV may be attenuated in younger animals.[26] Most studies used RT-PCR for measurement of ACE2 expression, with the exception of Nagata et al. who used IHC to localize ACE2.

Discussion

This review shows that higher levels of membrane-bound ACE2 facilitate worse outcomes. The outcomes measured in these studies included viral titres, inability to clear infection rapidly, higher inflammatory cytokines, and morbidity and mortality. These results confirm prior structural and phylogenetic studies on the necessity of ACE2 expression for SARS infectivity.[20,29,30] Notably, the included studies did not measure soluble ACE2 shedding in serum. It is therefore unknown whether injectable soluble ACE2 would hinder SARS infectivity based on these studies. However, our review provides insight into the fact that higher expression of membrane bound ACE2 increases viral load irrespective of soluble ACE2 levels. Future animal models should focus on administration of exogenous ACE2 protein and its subsequent effect on infectivity, as well as measurement of serum ACE2 in relation to viral infectivity.

These results are important in the context of renin-angiotensin-aldosterone system (RAAS)-modulating pharmacotherapy. Animal models seem to show mixed results on tissue ACE2 expression using both classical ACEis and ARBs.[31,32] In addition, a recent rapid review showed low certainty evidence from observational studies that patients on long-term therapy are not at higher risk of poorer outcomes.[33] However, these studies are merely observational in nature and do not address the potential histopathological changes that may occur with both medications and SARS infection. These results are also important in the context of the current COVID-19 pandemic. Recently, Mancia et al. showed that use of ARBs or ACEis did not show any association with COVID-19 in patients overall nor among those with severe disease.[34]

In addition, few studies differentiate between soluble and membrane-bound ACE2 expression. In multiple studies, plasma ACE2 activity was not higher among patients who were taking ACEis or ARBs than among untreated patients.[35-38] Notably, in a study on COVID-19, patients showed increased angiotensin II levels correlating with viral load and lung injury.[39] This could in theory result in increased ACE2 activity with increased substrate. Future studies should focus on systematically comparing ACEis' and ARBs' effect on ACE2 expression in order to guide clinical trials. In the context of our results, higher ACE2 expression would in theory facilitate higher infectivity.

Furthermore, the co-localization of ACE2 and SARS-CoV antigen suggests that the distribution of membrane-bound ACE2 dictates the histopathologic and clinical features of infection. In humans, ACE2 is found in alveolar type I and II cells more than epithelial cells in the upper respiratory tract.[11] It is found in enterocytes of the small intestines, consistent with the watery diarrhea commonly seen with SARS infection.[38] It is also found in

arterial and venous endothelium and arterial smooth muscles.[11] However, these tissues are not equally affected by ACEis. Hawng et al. found that captopril labelled with fluorine-18 distributed mostly to the lungs, kidney, and aorta.[39] As such, any effect of RAAS-modifying drugs on the infectivity of coronaviruses would likely have a larger impact on these organs. However, this is likely different depending on the specific drug, as Cushman et al. showed that different ACEis had different magnitude and duration of activity, determined by fluorimetry, in different rat tissues examined.[40]

ACE2 expression downregulation occurs following SARS-CoV infection; however, the degree of downregulation varies. Young mice showed less downregulation of ACE2; they also exhibited milder clinical symptoms compared to older mice. Additionally, more lethal strains of SARS-CoV were shown to have increased downregulation of ACE2. The exact cause of this downregulation is not known. Viral entry into cells could cause subsequent alterations in ACE2 mRNA transcription and protein translation. Thus ACE2 mRNA expression on RT-PCR may be decreased. On the other hand, coronavirus infection could cause destruction of susceptible cells with membrane-bound ACE2 receptors. Because fewer ACE2-expressing cells are present, the ACE2 levels measured would be decreased in more severe infections.

Conclusions

In conclusion, our review shows that increased expression of ACE2 facilitates further SARS infectivity and worse clinical outcome in animal models. ACE2 downregulation following viral infection is correlated with worse outcome; this may be due to decreased ACE2 expression or destruction of susceptible cells. Our study is limited by the lack of human trials on this topic.

PROSPERO registration

CRD42020176691

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