INTERNAL MEDICINE

Role of Angiotensin Converting Enzyme-2 and its modulation in disease: exploring new frontiers

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Abstract

Angiotensin Converting Enzyme-2 (ACE2), an important enzyme in the Renin Angiotensin Aldosterone System, degrades Angiotensin II (Ang II) into Angiotensin-(1-7) (Ang-(1-7)), whose actions are opposite to that of Ang II. Interestingly, SARS CoV-2 virus entry into human cells is mediated by ACE2. ACE2 receptors that are widely expressed in lungs and various other organs. Ang-(1-7) seems to have favorable effects on lungs, by preventing fibrosis in lung inflammation models, and exerts a similar action in cardiac and renal pathologies as well. Thus, modulation of Ang-(1-7) can be of potential benefit in chronic as well as acute inflammatory diseases affecting lungs and other organs. Upregulation of ACE2 by statins in different organs, and its consequent beneficial effects, have been demonstrated in many experimental studies, and also in a few clinical ones. This review aims at probing the role of ACE2 and its therapeutic modulation in pulmonary and extra pulmonary diseases, including COVID-19.

Keywords: Angiotensin II, COVID 19, HMG CoA reductase inhibitor, Renin -Angiotensin system

Introduction

The Renin Angiotensin Aldosterone System plays a vital role in the regulation of blood pressure, fluid and electrolyte balance, cardiac and renal function. Angiotensin Converting Enzyme (ACE), a zinc metallo-endopeptidase, is one of the key enzymes in this system. Angiotensin-Converting Enzyme 2 (ACE 2), a carboxypeptidase and a type I transmembrane glycoprotein, is the first known human homologue of ACE [1]. The catalytic domains of ACE and ACE2 are 42% identical in amino acid sequence [2], but they act on different substrates. ACE converts Angiotensin I (Ang I) to Angiotensin II (Ang II), which acts on Ang II type 1 receptor (AT_1R); while ACE 2 degrades Ang II into Angiotensin-(1-7) (Ang-(1-7)), which produces actions opposite to that of Ang II, as shown in figure 1.

Ang-(1-7) acts by binding to its functional receptor mas, a G-protein coupled receptor, first described as mas oncogene (the name has been derived from the surname of the patient whose tumor cells were used to identify the gene) [4]. ACE2 / Ang-(1-7)-mas receptor axis is primarily involved in vasodilation and anti-proliferative activity, while ACE / Ang-II-AT1 receptor is responsible for vasoconstriction, cardiac hypertrophy, and cellular proliferation. The actions of ACE2 counterbalance those of ACE by negatively regulating the level of Ang II [5]. This review explores the function of ACE2 in pulmonary and extra-pulmonary diseases and in COVID; also, it looks at the possible therapeutic role of ACE modulators in disease.

A PubMed search was performed and relevant articles related to role in ACE2 in pulmonary, extra-pulmonary disease and in COVID were included in this review.

ACE2 in pulmonary diseases

ACE2 receptors are widely expressed in lungs, heart, kidney, gut, vessels, testis and brain. Increased

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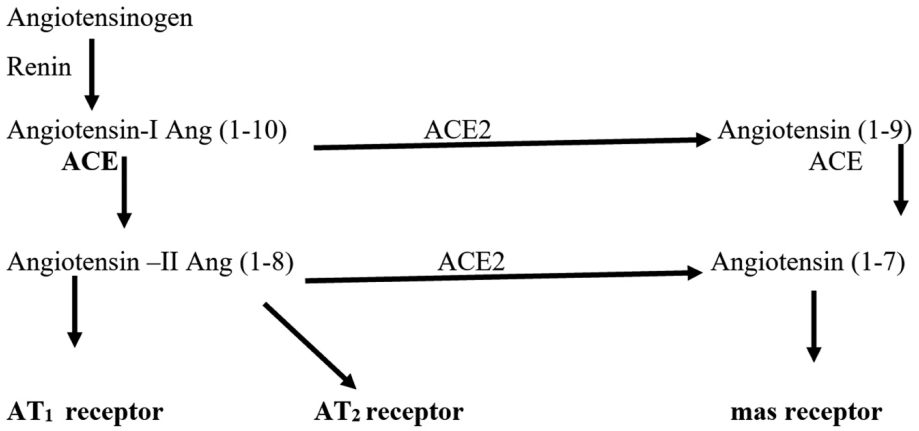

Figure 1. Renin-Angiotensin-mas receptor cascade [3].

expression of ACE in lung capillaries generates a large amount of Ang II in the lungs [6]. Ang II in turn produces pulmonary vasoconstriction mediated by AT_1R, thereby increasing the vascular tone and causing pulmonary edema [7]. On the other hand, Ang-(1-7) appears to have beneficial effects on the lungs, preventing fibrosis in lung inflammation models by inhibiting central mediators like ERK1/2, p38, JNK [8]. Interestingly, this inhibition has been observed in lung cancer cells as well [9]. Anti-inflammatory action of Ang-(1-7) is also mediated by decreasing TGF-β/NFκB signaling pathway [10] and other pro-inflammatory molecules. This anti-inflammatory action of Ang-(1-7) has been demonstrated in other lung pathologies, like bronchial asthma [10]. In alveolar epithelial cell (AEC) lines, ACE2 regulates AEC survival by balancing the pro-apoptotic Ang II and anti-apoptotic Ang-(1-7), with the latter acting through Ang-(1-7) mas [11].

In chronic ovalbumin challenged mice, treatment with Ang-(1-7), by acting through mas receptors, prevented pulmonary remodeling, bronchial hyper-responsiveness and release of pro-inflammatory cytokines. Prevention of remodeling was associated with suppression of phosphorylation of ERK1/2 and JNK dependent pathways by Ang-(1-7) [12]. Thus, therapeutic modulation of Ang-(1-7) in lungs can be potentially useful in chronic inflammatory disease of lungs [12]. Lung specimens from patients with idiopathic pulmonary fibrosis revealed that ACE2 mRNA and ACE2 activity in these specimens was reduced by 92% [13]. Similar results were also seen in experimental models of lung fibrosis induced by bleomycin in rats where the ACE2 was downregulated. On the other hand, systemic administration of ACE2 reduced bleomycin-mediated collagen deposition to levels comparable to normal rats in the control group. Authors suggested that high ACE 2 expression limits the local production of Ang II in lungs, thereby having a protective role in pulmonary fibrosis [13]. The protective role of ACE2 has also been demonstrated in pulmonary arterial hypertension and is hypothesized to be brought about by attenuating pulmonary oxidative stress and preventing vascular impairment, and cardiac remodeling [14]. Researchers have developed a system to generate ACE2 within chloroplasts using tansplastomic technology, which on oral administration reduces pulmonary hypertension in rats [15]. In lung pathologies associated with pulmonary hypertension, it has been found that XNT offsets the development of pulmonary hypertension by shifting the balance from vasoconstrictive to vasoprotective axis in renin angiotensin axis [16].

Three experimental models of acute lung injury, i.e. acid aspiration lung injury, endotoxin-induced ARDS and peritoneal sepsis-induced ARDS, were used to investigate the protective role of ACE2 in the gene knockout mice. The *ace2* gene knockout mice showed deterioration in lung biology manifested as neutrophil accumulation and lung edema, while *ace* mutant mice had better lung state. In wild-type mice, acid aspiration downregulated ACE2 expression while the levels of ACE remained constant, with increased Ang II levels in lungs and plasma. Administration of recombinant ACE2 protein decreased Ang II levels in the lungs of these acid-treated mice, thus reducing lung injury. In another experimental model of ARDS with reduction of ACE2 and a simultaneous fall in Ang-(1-7) activity, administration of protease-resistant cyclic form of Ang-(1–7) [cAng-(1–7)] halted the progression of ARDS, by inhibiting the production of inflammatory mediators. This reduced the lung injury scores and improved oxygenation [17]. These findings elucidate the protective role of ACE2 in ARDS [13] and in other types of lung injury. On the other hand, ACE, Ang II, and AT_1R axis promote lung injury [18].

In addition, ACE2 modifies other bioactive peptides, such as kinins. These are autacoids involved in cardiovascular function, inflammation and pain. Their effects are mediated by B1 and B2 receptors. The active metabolite of bradykinin, des-Arg9 bradykinin (DABK), is a substrate for ACE2

in airway epithelia. ACE2 removes a single amino acid from DABK and inhibits DABK/ Bradykinin B1 receptor (BKB1R) activation. Deficiency of ACE2 in lungs induced by endotoxins leads to impaired inactivation of DABK and increased activity of DABK/BKB1R axis which promotes infiltration of neutrophils into the lungs, leading to acute lung inflammation [19]. This pro inflammatory action is mediated by the production and release of chemokines like CXCL5 from airway epithelial cells [19].

The beneficial effects of ACE2 in chronic lung illnesses and ARDS can be exploited for therapeutic purposes. Thus, drugs that augment ACE2 activity (stimulating the expression of ACE2 or administration of recombinant ACE2) may have a potential therapeutic role in the management of various lung pathologies [3,20].

ACE2 in extra-pulmonary diseases

ACE2 receptors are also present in endothelial cells, smooth muscle cells and cardiac myocytes [21]. Ang-(1-7) by acting on mas receptors produces actions like vasodilation, inhibition of cell growth, anti-thrombosis, etc [22]. On platelets, Ang-(1-7) produces an antithrombotic effect by increasing NO (nitric oxide) and prostacyclins [23]. Antithrombotic effect of Ang-(1-7) involves the release of NO from platelets and endothelial cells mediated by mas receptors. Investigators have suggested that Ang-(1-7)- mas axis can be a potential target for management of thrombotic diseases [24]. Pretreatment with orally active formulation of Ang-(1-7) produced antithrombotic effect in spontaneously hypertensive rats [25]. Also, earlier studies have shown that antithrombotic effects of ACE inhibitors and ARBs are mediated by Ang-(1-7) in renal hypertensive rats [26]. Among the different cardiac cell types, predominant ACE2 expression is in pericytes [27].

Chronic administration of Ang-(1-7) reduces cardiac hypertrophy and fibrosis, and preserves left ventricular function without change in Ang-II levels in experimental models of suprarenal abdominal aortic coarctation [28]. Downregulation of ACE2 / Ang-(1-7)- mas receptor axis, reported in cusps of the aortic valve in humans, produces fibrosis and inflammation seen in patients with aortic stenosis [29]. The beneficial effects of Ang-(1-7) in heart involves reducing fibrosis, decreasing the infarction area and protecting cardiac remodeling.

In renal injury as well, ACE2 appears to have a protective influence [30]. ACE2 expression is decreased in hypertensive rats in comparison to normotensive controls [31]. The reno-protective effect of ACE2 is supposed to be mediated by Ang–II degradation; thus the deficiency or inhibition of ACE2 can lead to kidney injury and dysfunction [32]. Again, in another experimental model, it was found that in rats with chronic kidney disease induced by subtotal nephrectomy, renal ACE2 deficiency contributed to the renal tissue injury [33]. Upregulation or replenishing of ACE2 can be useful in such conditions, and this was confirmed by a study which found that ACE activator caused reduction in blood pressure and reversal of cardiac and renal fibrosis in spontaneously hypertensive rat model of hypertension [34]. The role of ACE2 activators as anti-hypertensives and in reversing cardiac and renal fibrosis has also been suggested [34]. High levels of ACE2 are also found in the gastrointestinal tract: ileum, duodenum, jejunum, caecum and colon [35].

In view of the above evidence, drugs that potentiate the action of ACE2/ Ang-(1-7)-mas axis may be useful in chronic diseases with inflammatory, fibrotic and proliferative components [36].

Various studies investigating the role of ACE2 in pulmonary and extrapulmonary diseases have been summarized in table I.

ACE2 in COVID-19

Coronaviruses, SARS-CoV-1 and SARS-CoV-2, require host ACE2 as a co-receptor in order to have intracellular penetration into lungs [37]. This fact has generated a lot of interest in pulmonary ACE2 expression amidst raging COVID-19 pandemic [38]. The viral envelope of coronaviruses has structural proteins, membrane proteins and envelope proteins involved in the viral assembly, and it is the SPIKE protein S which mediates the entry of the virus into the host cell [39].

The SPIKE protein S is cleaved into S1 and S2 subunits by host protease, TMPRSS2 (transmembrane protease serine 2). Viral attachment to target cells in the pulmonary epithelium is facilitated by the S1 subunit, while S2 subunit is responsible for membrane fusion and internalization of the virus along with ACE2 [37]. An inhibitor of TMPRSS2, camostat mesylate, blocks the entry of SARS-CoV-2 and SARS-CoV-1 into the cells [37]. The expression of ACE2 on the cell surface is significantly reduced secondary to internalization and shedding (cleavage of extracellular juxta region of ACE2) [40]. With ACE2 internalization, the expression of ACE2 at cell surface is lost, thereby slowing down the conversion of Ang II to the protective Ang-(1-7). Increase in the ratio of Ang II to Ang-(1-7) worsens the pulmonary damage provoked by SARS-CoV-2 [41]. Ang II mediates tissue inflammation via macrophages, dendritic cells, T-cells, mesangial cells and vascular smooth muscle cells [42]. Increased vascular permeability, a hallmark in pathogenesis of ARDS, is accentuated in mice with ACE2 deficiency [43]. Elderly persons and those with comorbidities such as hypertension and diabetes mellitus are at increased risk of severe COVID-19 disease, and also higher mortality [44,45]. It has been observed that in individuals with these clinical conditions, there is a deficiency of ACE2. Moreover, in COVID-19 the binding of virus to ACE2 receptors, membrane fusion, viral entry into the cell and the subsequent downregulation of these receptors favor progression of inflammation and thrombosis [46], to the detriment of the population at risk.

Table I. Summary of animal and human studies exploring the role of ACE2 in disease.

Study	Year	Methodology	Conclusion
Animal Studies			
Role of ACE2 in pulmonary diseases			
Santos et al. [60]	2018	The effect of atorvastatin alone or in combination with ischemic post conditioning in the prevention of reperfusion injury in the lungs of Wistar Norvegic rats subjected to ischemia and reperfusion by aortic clamping was studied.	Atorvastatin alone and in combination with ischemic postconditioning minimized reperfusion injury.
Yu et al. [61]	2017	In two groups of C57BL/6 mice either lipopolysaccharides (LPS) alone or lipopolysaccharide with simvastatin was administered.	Protective effects of simvastatin was observed on LPS-induced lung injury and this action was mediated by stabilizing cytoskeleton and intercellular adherent junctions.
Mathew et al. [64]	2011	The effect of simvastatin was assessed in murine model of radiation induced lung injury (RILI).	Simvastatin reduced RILI indices like vascular leakage, leucocyte infiltration and oxidative stress.
Wösten-van Asperen et al. [17]	2011	Intratracheal LPS-induced rat model of ARDS was administered either saline as placebo, losartan or cyclic form of Ang-(1-7).	Administration of Ang 1-7 countered the development of ARDS.
Ferreira et al. [16]	2011	In rat model of pulmonary hypertension the mechanism underlying the protective effects of an ACE activator was studied.	ACE activator stimulates cardiac ACE2 and thereby inhibits cardiac fibrosis.
Li et al. [13]	2008	ACE-2 siRNA was administered in bleomycin-treated rats and 57-BL6 mice.	ACE-2 siRNA administration increased the ANG II level of lung tissue.
Role of ACE2 in extra-pulmonary diseases			
Shin et al. [59]	2017	Male wistar rats were studied as control group, diabetes group, diabetes plus insulin group and diabetes plus insulin plus fluvastatin group.	In diabetic rats, fluvastatin produced a beneficial effect on myocardial fibrosis and ACE2 expression.
Li et al. [56]	2013	Wistar rats models of intimal thickening after balloon injury were divided into three groups: control, surgery and rosuvastatin.	Rosuvatatin had an inhibitory effect on thickening by upregulating ACE2, increasing Ang 1-7 and downregulating AT1.
Fraga-Silva et al. [25]	2011	Thrombus formation was induced in spontaneously hypertensive rats and the antithrombotic effect of cyclodextrin [Ang-(1-7)-CyD] was tested.	Oral administration of cyclodextrin [Ang-(1-7)- CyD] produced Mas dependent antithrombotic effect.
Fraga-Silva et al. [24]	2008	In rat and mouse platelets Western blot was done to test the presence of Mas proteins.	Western-blotting revealed a single band corresponding to the Mas protein in protein extracts prepared from isolated rat platelets. It was proposed that the antithrombotic effect of Ang 1-7 was mediated by Mas mediated NO release.
Kang et al. [58]	2008	Spontaneous hypertensive models of rats were divided into two groups one group was treated with atorvastatin and other with distilled water for 10 weeks.	Atorvastain upregulated the expression of p27 mRNA and facilitated cardiomyocytes apoptosis and thus regulated ventricular hypertrophy.
Soler et al. [32]	2007	In Streptozotocin-induced diabetic mice, a specific ACE 2 inhibitor MLN-4760 was given, and the effect was compared with normal mice.	Administration of ACE 2 inhibitor to diabetic mice chronically worsened glomerular injury.
Wang et al. [28]	2005	In rats with experimentally-induced suprarenal abdominal aortic coarctation, the effect of chronic administration of Ang- (1-7) was evaluated.	Ang 1-7 reduced cardiac hypertrophy and fibrosis.
Clinical studies			
Li et al. [65]	2021	ACE2 and TMPRSS2 expression levels in lung tissues of control non-Idiopathic pulmonary fibrosis and Idiopathic pulmonary fibrosis patients were measured.	There was upregulation of ACE2 and TMPRSS2 in pulmonary fibrosis
Peymani et al. [63]	2021	In a retrospective study of Iranian patients (n=150) with COVID-19, an association between statin use and mortality was evaluated.	Statin use was associated with a significant reduction in mortality in patients with COVID-19.
Chen et al. [27]	2020	Study was done on human heart tissues obtained from donors in a transplantation centre.	In adult human heart, ACE2 is highly expressed in pericytes and patients with heart failure have increased expression of ACE2 and this provides an modality to treat cardiac injury
Hemnes et al. [68]	2018	A phase IIa, open-label pilot study was done to determine the effects of single infusion of rhACE2 in patients with pulmonary hypertension.	rhACE2 increased cardiac output significantly, also a decreased ratio of AngII/ Ang-(1-7) suggested an increase in ACE2 activity.
Khan et al. [67]	2017	In a phase II RCT in North America, 10 patients with diagnosis of ARDS were administered recombinant form of human ACE2 (rhACE2).	Ang II levels decreased following infusion of recombinant ACE2 (rhACE2) and Ang-(1-7) remained increased for 48 hours.
Mizuiri et al. [66]	2008	Kidney tissues were taken from 20 patients with type 2 diabetes and overt nephropathy and 20 healthy kidney donors, and renal expression of ACE and ACE2 was assessed using immunohistochemistry and insitu hybridization.	Significant decrease in ACE2 and an increase in ACE was observed in diabetics with overt nephropathy.
Li et al. [13]	2008	ACE2 assay was done in lung biopsy specimens from patients with idiopathic pulmonary fibrosis	ACE-2 by preventing increase of profibrotic peptide ANG II prevents the lung fibrogenesis

In the central nervous system, high ACE2 expression has been found in areas like substantia nigra and ventricles in human and mice brain. This can provide a potential pathway for SARS CoV 2 virus to enter CSF or spread around brain [47].

COVID-19 increases the risk of cardiovascular diseases and more so in patients with genetically elevated cholesterol levels [48]. As ACE2 is involved in homeostatic processes, prevention of thrombus formation and attachment of platelets to vessel wall, ACE2 activator 1-[(2-dimethylamino) ethylamino]-4-(hydroxymethyl)-7-[(4-methylphenyl) sulfonyl oxy]-9H-xanthene-9-one (XNT) can be a lead compound in the treatment of thrombogenesis [49], and may find a use in COVID-19 patients as well [46]. Therapeutic approaches that upregulate ACE 2 can perhaps be one of the possible ways to halt COVID progression.

Various clinical studies including systematic reviews, meta-analyses, and observational studies have found that ARB or ACEI did not adversely impact disease severity or mortality in patients with COVID-19. These studies seem to support the consensus view that these drugs need not be stopped in patients who have been on these prior to contracting COVID-19 infection. [50,51,52]. However, certain individual patient factors like ACE2 polymorphisms, having a potential to increase the risk of unfavorable outcomes, need to be probed further [53].

Interestingly, a study of veterans with hypertension revealed that ACEI was significantly associated with reduced odds of testing positive for COVID-19. In this study too, ARB nor ACEI use was not associated with any adverse clinical outcomes in COVID-19-positive subjects. There was, however, an association of ACEI with a small increase in the odds of mechanical ventilation need, though not clinically significant [54].

Potential role of statins in ACE2 modulation

The pleiotropic effects of statins provide endothelial stability, antioxidant activity, anti-inflammatory properties and stabilization of atherosclerotic plaques. These actions are mediated by inhibiting isoprenylation of small GTPases, which in turn inhibits the downstream signaling molecules Rho Rac [55].

In experimental models, statins have been shown to modulate various processes like inflammation and fibrinogenesis. Using the model of balloon catheter aortic endothelial denudation in wistar rats, it was found that the decreased expression of ACE2 caused a consequent increase in levels of Ang II, leading to proliferation and migration of vascular smooth muscle cells and intimal hyperplasia [51]. On administering rosuvastatin in these models, there was reduced intimal proliferation—which could be attributed to increased level of Ang (1-7)— and also downregulation of AT1, upregulation of ACE2 and activation of P-ERK pathway [56].

Experimental and clinical studies have demonstrated the protective effect of statins on the lungs. ACE-2/ Ang-(1-7)/mas signaling axis prevents ARDS in acute lung injury. In rabbit model of atherosclerosis, atorvastatin administration for three weeks increased ACE2 expression in the heart by histone modification [57]. In left ventricular hypertrophy model in rat, atorvastatin reduced hypertrophy which has been partly attributed to reduced ACE2 mRNA and ACE2 protein expression [6]. Atorvastatin by upregulating the expression of mRNA and protein levels of p27 controls ventricular hypertrophy in spontaneously hypertensive rat models [58]. In Streptozotocin-induced diabetic rat models, fluvastatin and insulin combination was more effective than insulin alone in attenuating cardiac fibrosis. Also the investigators found that ACE2 expression was restored in the combination group [59]. ACE2 activation affords protection against diabetes-induced cardiac dysfunction and hypertensive myocardial fibrosis. Thus, it has been proposed that the role of ACE2 activator should be investigated in the treatment of cardiac fibrosis [16]. In the lungs of rats subjected to ischemia and reperfusion by aortic clamping, atorvastatin prevented reperfusion injury. The authors suggested that statins, by inhibiting HMGCoA reductase, suppress the synthesis of isoprenoids, which are ligands for posttranslational modification of proteins like Rho, Ras, Rac, and thereby prevent lung injury [60]. Simvastatin in liposaccharide-induced acute lung injury murine model, produced a protective effect by stabilizing cytoskeleton and adherens junction proteins, which are important components of intercellular junction [61]. Statins by modulating immune response and controlling cytokine expression can prevent ARDS [62]. Activation of autophagy-associated signaling in lung epithelial cells is considered to be the molecular mechanism by which statins produce their protective effect in viral infections [63]. In radiation-induced lung injury (RILI) in mice, simvastatin reduced RILI inflammatory markers like lung permeability, leukocyte infiltration, and pro-inflammatory cytokine secretion. Hence, such drugs have a potential role in permitting escalation of therapeutic radiation dose, thereby improving patient outcomes in thoracic malignancies [64]. In addition to activating ACE2, statins have been found to act through other non-lipid lowering mechanisms by which they have a protective role in various conditions.

Strengths

This review points towards an emerging body of evidence which can open up a new frontier, for exploration into disease mitigation via ACE 2 modulation.

Limitations

Paucity of well-designed clinical studies which demonstrate a clear cut improvement in clinical outcomes puts a limit on the use of ACE2 modulation in clinical practice.

Conclusion

ACE2 plays an important role in the pathogenesis of many pulmonary and extra pulmonary diseases, including COVID-19. Downregulation of ACE2 produces an adverse inflammatory and immune response in such conditions, and these can potentially be offset by restoring the balance between ACE and ACE2 levels. Thus, therapeutic strategies that upregulate ACE2 can slow the progression of many pathologies, as has been demonstrated by use of statins and ACE2 activators. The anti-inflammatory, antithrombotic and immunomodulatory actions of statins have been found to be of potential benefit in COVID-19, as well.

References

1. Toklu HZ, Kwon OS, Sakarya Y, Powers SK, Llinas K, Kirichenko N, et al. The effects of enalapril and losartan on mechanical ventilation-induced sympathoadrenal activation and oxidative stress in rats. J Surg Res. 2014;188:510–516.
2. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, et al. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. Circ Res. 2000;87:E1– E9.
3. Hamming I, Cooper ME, Haagmans BL, Hooper NM, Korstanje R, Osterhaus AD, et al. The emerging role of ACE2 in physiology and disease. J Pathol. 2007;212:1-11.
4. Young D, Waitches G, Birchmeier C, Fasano O, Wigler M. Isolation and characterization of a new cellular oncogene encoding a protein with multiple potential transmembrane domains. Cell. 1986;45:711-719.
5. Imai Y, Kuba K, Penninger JM. Angiotensin-converting enzyme 2 in acute respiratory distress syndrome. Cell Mol Life Sci. 2007;64:2006–2012.
6. Studdy PR, Lapworth RU, Bird R. Angiotensin-converting enzyme and its clinical significance--a review. J Clin Pathol. 1983;36:938-947.
7. Kiely DG, Cargill RI, Wheeldon NM, Coutie WJ, Lipworth BJ. Haemodynamic and endocrine effects of type 1 angiotensin II receptor blockade in patients with hypoxaemic cor pulmonale. Cardiovasc Res. 1997;33:201-208.
8. Santos RAS, Sampaio WO, Alzamora AC, Motta-Santos D, Alenina N, Bader M, et al. The ACE2/Angiotensin-(1-7)/MAS Axis of the Renin-Angiotensin System: Focus on Angiotensin-(1-7). Physiol Rev. 2018;98:505-553.
9. Ni L, Feng Y, Wan H, Ma Q, Fan L, Qian Y, et al. Angiotensin-(1-7) inhibits the migration and invasion of A549 human lung adenocarcinoma cells through inactivation of the PI3K/Akt and MAPK signaling pathways. Oncol Rep. 2012;27:783–790.
10. El-Hashim AZ, Renno WM, Raghupathy R, Abduo HT, Akhtar S, Benter IF. Angiotensin-(1-7) inhibits allergic inflammation, via the MAS1 receptor, through suppression of ERK1/2- and NF-κB-dependent pathways. Br J Pharmacol. 2012;166:1964–1976.
11. Uhal BD, Li X, Xue A, Gao X, Abdul-Hafez A. Regulation of alveolar epithelial cell survival by the ACE-2/angiotensin 1-7/Mas axis. Am J Physiol Lung Cell Mol Physiol. 2011;301:L269-L274.
12. Magalhães GS, Rodrigues-Machado MG, Motta-Santos D, Silva AR, Caliari MV, Prata LO et al. Angiotensin-(1-7) attenuates airway remodelling and hyperresponsiveness in a model of chronic allergic lung inflammation. Br J Pharmacol. 2015;172:2330-2342.
13. Li X, Molina-Molina M, Abdul-Hafez A, Uhal V, Xaubet A, Uhal BD. Angiotensin converting enzyme-2 is protective but downregulated in human and experimental lung fibrosis. Am J Physiol Lung Cell Mol Physiol. 2008;295:L178–L185.
14. Shenoy V, Qi Y, Katovich MJ, Raizada MK. ACE2, a promising therapeutic target for pulmonary hypertension. Curr Opin Pharmacol. 2011;11:150-155.
15. Shenoy V, Kwon KC, Rathinasabapathy A, Lin S, Jin G, Song C, et al. Oral delivery of Angiotensin-converting enzyme 2 and Angiotensin-(1-7) bioencapsulated in plant cells attenuates pulmonary hypertension [published correction in Hypertension. 2015;65:e8]. Hypertension. 2014;64:1248-1259.
16. Ferreira AJ, Shenoy V, Qi Y, Fraga-Silva RA, Santos RA, Katovich MJ, et al. Angiotensin-converting enzyme 2 activation protects against hypertension-induced cardiac fibrosis involving extracellular signal-regulated kinases. Exp Physiol. 2011;96:287-294.
17. Wösten-van Asperen RM, Lutter R, Specht PA, Moll GN, van Woensel JB, van der Loos CM, et al. Acute respiratory distress syndrome leads to reduced ratio of ACE/ACE2 activities and is prevented by angiotensin-(1-7) or an angiotensin II receptor antagonist. J Pathol. 2011;225:618-627.
18. Imai Y, Kuba K, Penninger JM. The discovery of angiotensin-converting enzyme 2 and its role in acute lung injury in mice. Exp Physiol. 2008;93:543–548.
19. Sodhi CP, Wohlford-Lenane C, Yamaguchi Y, Prindle T, Fulton WB, Wang S, et al. Attenuation of pulmonary ACE2 activity impairs inactivation of des-Arg^9 bradykinin/BKB1R axis and facilitates LPS-induced neutrophil infiltration. Am J Physiol Lung Cell Mol Physiol. 2018;314:L17–L31.
20. Cheng H, Wang Y, Wang GQ. Organ-protective effect of angiotensin-converting enzyme 2 and its effect on the prognosis of COVID-19. J Med Virol. 2020;92:726-730.
21. Burrell LM, Risvanis J, Kubota E, Dean RG, MacDonald PS, Lu S, et al. Myocardial infarction increases ACE2 expression in rat and humans. Eur Heart J. 2005;26:369–375; discussion 322-324.
22. Schindler C, Bramlage P, Kirch W, Ferrario CM. Role of the vasodilator peptide angiotensin-(1-7) in cardiovascular drug therapy. Vasc Health Risk Manag. 2007;3:125-137.
23. Fang C, Stavrou E, Schmaier AA, Grobe N, Morris M, Chen A, et al. Angiotensin 1-7 and Mas decrease thrombosis in Bdkrb2-/- mice by increasing NO and prostacyclin to reduce platelet spreading and glycoprotein VI activation. Blood. 2013;121:3023–3032.
24. Fraga-Silva RA, Pinheiro SV, Gonçalves AC, Alenina N, Bader M, Santos RA. The antithrombotic effect of angiotensin-(1-7) involves mas-mediated NO release from platelets. Mol Med. 2008;14:28-35.

25. Fraga-Silva RA, Costa-Fraga FP, De Sousa FB, Alenina N, Bader M, Sinisterra RD, et al. An orally active formulation of angiotensin-(1-7) produces an antithrombotic effect. Clinics (Sao Paulo). 2011;66:837-841.
26. Kucharewicz I, Pawlak R, Matys T, Pawlak D, Buczko W. Antithrombotic effect of captopril and losartan is mediated by angiotensin-(1-7). Hypertension. 2002;40:774-779.
27. Chen L, Li X, Chen M, Feng Y, Xiong C. The ACE2 expression in human heart indicates new potential mechanism of heart injury among patients infected with SARS-CoV-2. Cardiovasc Res. 2020;116:1097-1100.
28. Wang LJ, He JG, Ma H, Cai YM, Liao XX, Zeng WT, et al. Chronic administration of angiotensin-(1-7) attenuates pressure-overload left ventricular hypertrophy and fibrosis in rats. Di Yi Jun Yi Da Xue Xue Bao. 2005;25:481-487.
29. Peltonen T, Näpänkangas J, Ohtonen P, Aro J, Peltonen J, Soini Y, et al. (Pro)renin receptors and angiotensin converting enzyme 2/angiotensin-(1-7)/Mas receptor axis in human aortic valve stenosis. Atherosclerosis. 2011;216:35-43.
30. Tikellis C, Bialkowski K, Pete J, Sheehy K, Su Q, Johnston C, et al ACE2 deficiency modifies renoprotection afforded by ACE inhibition in experimental diabetes. Diabetes. 2008;57:1018-1025.
31. Zhong JC, Huang DY, Yang YM, Li YF, Liu GF, Song XH, et al. Upregulation of angiotensin-converting enzyme 2 by all-trans retinoic acid in spontaneously hypertensive rats. Hypertension. 2004;44:907-912.
32. Soler MJ, Wysocki J, Ye M, Lloveras J, Kanwar Y, Batlle D. ACE2 inhibition worsens glomerular injury in association with increased ACE expression in streptozotocin-induced diabetic mice. Kidney Int. 2007;72:614-623.
33. Burrell LM, Burchill L, Dean RG, Griggs K, Patel SK, Velkoska E. Chronic kidney disease: cardiac and renal angiotensin-converting enzyme (ACE) 2 expression in rats after subtotal nephrectomy and the effect of ACE inhibition. Exp Physiol. 2012;97:477-485.
34. Hernández Prada JA, Ferreira AJ, Katovich MJ, Shenoy V, Qi Y, Santos RA, et al. Structure-based identification of small-molecule angiotensin-converting enzyme 2 activators as novel antihypertensive agents. Hypertension. 2008;51:1312-1317.
35. Harmer D, Gilbert M, Borman R, Clark KL. Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. FEBS Lett. 2002;532:107-110.
36. Simões e Silva AC, Silveira KD, Ferreira AJ, Teixeira MM. ACE2, angiotensin-(1-7) and Mas receptor axis in inflammation and fibrosis. Br J Pharmacol. 2013;169:477-492.
37. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 2020;181:271-280.e8.
38. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, et al Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003;426:450-454.
39. Li F. Structure, Function, and Evolution of Coronavirus Spike Proteins. Annu Rev Virol. 2016;3:237-261.
40. Liu M, Wang T, Zhou Y, Zhao Y, Zhang Y, Li J. Potential Role of ACE2 in Coronavirus Disease 2019 (COVID-19) Prevention and Management. J Transl Int Med. 2020;8:9-19.
41. South AM, Diz DI, Chappell MC. COVID-19, ACE2, and the cardiovascular consequences. Am J Physiol Heart Circ Physiol. 2020;318:H1084-H1090.
42. Benigni A, Cassis P, Remuzzi G. Angiotensin II revisited: new roles in inflammation, immunology and aging. EMBO Mol Med. 2010;2:247-257.
43. Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature. 2005;436:112–116.
44. Li JY, You Z, Wang Q, Zhou ZJ, Qiu Y, Luo R, et al. The epidemic of 2019-novel-coronavirus (2019-nCoV) pneumonia and insights for emerging infectious diseases in the future. Microbes Infect. 2020;22:80-85.
45. Du RH, Liang LR, Yang CQ, Wang W, Cao TZ, Li M, et al. Predictors of mortality for patients with COVID-19 pneumonia caused by SARS-CoV-2: a prospective cohort study. Eur Respir J. 2020;55:2000524.
46. Verdecchia P, Cavallini C, Spanevello A, Angeli F. The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. Eur J Intern Med. 2020;76:14-20.
47. Chen R, Wang K, Yu J, Howard D, French L, Chen Z, et al. The Spatial and Cell-Type Distribution of SARS-CoV-2 Receptor ACE2 in the Human and Mouse Brains. Front Neurol. 2021;11:573095.
48. Vuorio A, Watts GF, Kovanen PT. Familial hypercholesterolaemia and COVID-19: triggering of increased sustained cardiovascular risk. J Intern Med. 2020;287:746-747.
49. Fraga-Silva RA, Sorg BS, Wankhede M, Dedeugd C, Jun JY, Baker MB, et al. ACE2 activation promotes antithrombotic activity. Mol Med. 2010;16:210-215.
50. Dai XC, An ZY, Wang ZY, Wang ZZ, Wang YR. Associations Between the Use of Renin-Angiotensin System Inhibitors and the Risks of Severe COVID-19 and Mortality in COVID-19 Patients With Hypertension: A Meta-Analysis of Observational Studies. Front Cardiovasc Med. 2021;8:609857.
51. Derington CG, Cohen JB, Mohanty AF, Greene TH, Cook J, Ying J, et al. Angiotensin II receptor blocker or angiotensin-converting enzyme inhibitor use and COVID-19-related outcomes among US Veterans. PLoS One. 2021;16:e0248080.
52. Wang W, Zhao X, Wei W, Fan W, Gao K, He S, et al. Angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARBs) may be safe for COVID-19 patients. BMC Infect Dis. 2021;21:114.
53. Grover A, Oberoi M. A systematic review and meta-analysis to evaluate the clinical outcomes in COVID-19 patients on angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. Eur Heart J Cardiovasc Pharmacother. 2021;7:148-157.
54. Li M, Wang Y, Ndiwane N, Orner MB, Palacios N, Mittler B, et al. The association of COVID-19 occurrence and

severity with the use of angiotensin converting enzyme inhibitors or angiotensin-II receptor blockers in patients with hypertension. PLoS One. 2021;16:e0248652.

55. Xiao H, Qin X, Ping D, Zuo K. Inhibition of Rho and Rac geranylgeranylation by atorvastatin is critical for preservation of endothelial junction integrity. PLoS One. 2013;8:e59233.
56. Li YH, Wang QX, Zhou JW, Chu XM, Man YL, Liu P, et al. Effects of rosuvastatin on expression of angiotensin-converting enzyme 2 after vascular balloon injury in rats. J Geriatr Cardiol. 2013;10:151-158.
57. Tikoo K, Patel G, Kumar S, Karpe PA, Sanghavi M, Malek V, et al. Tissue specific up regulation of ACE2 in rabbit model of atherosclerosis by atorvastatin: role of epigenetic histone modifications. Biochem Pharmacol. 2015;93:343-351.
58. Kang L, Hu SJ. Effects of atorvastatin on p27 protein expression and cardiomyocytes apoptosis in spontaneously hypertensive rats. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2008;24:46-49.
59. Shin YH, Min JJ, Lee JH, Kim EH, Kim GE, Kim MH, et al. The effect of fluvastatin on cardiac fibrosis and angiotensin-converting enzyme-2 expression in glucose-controlled diabetic rat hearts. Heart Vessels. 2017;32:618-627.
60. Santos CHMD, Dourado DM, Silva BAKD, Pontes HBD, Azevedo Neto E, Vendas GSDC, et al. Effect of Ischemic Postconditioning and Atorvastatin in the Prevention of Remote Lung Reperfusion Injury. Braz J Cardiovasc Surg. 2018;33:115-121.
61. Yu Y, Jing L, Zhang X, Gao C. Simvastatin Attenuates Acute Lung Injury via Regulating CDC42-PAK4 and Endothelial Microparticles. Shock. 2017;47:378-384.
62. Liberale L, Carbone F, Montecucco F, Sahebkar A. Statins reduce vascular inflammation in atherogenesis: A review of underlying molecular mechanisms. Int J Biochem Cell Biol. 2020;122:105735.
63. Peymani P, Dehesh T, Aligolighasemabadi F, Sadeghdoust M, Kotfis K, Ahmadi M, et al. Statins in patients with COVID-19: a retrospective cohort study in Iranian COVID-19 patients. Transl Med Commun. 2021;6:3.
64. Mathew B, Huang Y, Jacobson JR, Berdyshev E, Gerhold LM, Wang T, et al. Simvastatin attenuates radiation-induced murine lung injury and dysregulated lung gene expression. Am J Respir Cell Mol Biol. 2011;44:415-422.
65. Li HH, Liu CC, Hsu TW, Lin JH, Hsu JW, Li AF, et al. Upregulation of ACE2 and TMPRSS2 by particulate matter and idiopathic pulmonary fibrosis: a potential role in severe COVID-19. Part Fibre Toxicol. 2021;18:11.
66. Mizuiri S, Hemmi H, Arita M, Ohashi Y, Tanaka Y, Miyagi M, et al. Expression of ACE and ACE2 in individuals with diabetic kidney disease and healthy controls. Am J Kidney Dis. 2008;51:613-623.
67. Khan A, Benthin C, Zeno B, Albertson TE, Boyd J, Christie JD, et al. A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. Crit Care. 2017;21:234.
68. Hemnes AR, Rathinasabapathy A, Austin EA, Brittain EL, Carrier EJ, Chen X, et al. A potential therapeutic role for angiotensin-converting enzyme 2 in human pulmonary arterial hypertension. Eur Respir J. 2018;51:1702638.