The ACE-2 in COVID-19: Foe or Friend?

ABSTRACT

COVID-19 is a rapidly spreading outbreak globally. Emerging evidence demonstrates that older individuals and people with underlying metabolic conditions of diabetes mellitus, hypertension, and hyperlipidemia are at higher risk of morbidity and mortality. The SARS-CoV-2 infects humans through the angiotensin converting enzyme (ACE-2) receptor. The ACE-2 receptor is a part of the dual system renin-angiotensin-system (RAS) consisting of ACE-Ang-II-AT1R axis and ACE-2-Ang-(1–7)-Mas axis. In metabolic disorders and with increased age, it is known that there is an upregulation of ACE-Ang-II-AT1R axis with a downregulation of ACE-2-Ang-(1–7)-Mas axis. The activated ACE-Ang-II-AT1R axis leads to pro-inflammatory and pro-fibrotic effects in respiratory system, vascular dysfunction, myocardial fibrosis, nephropathy, and insulin resistance. On the other hand, the ACE-2-Ang-(1–7)-Mas axis has anti-inflammatory and antifibrotic effects on the respiratory system and anti-inflammatory, antioxidative stress, and protective effects on vascular function, protects against myocardial fibrosis, nephropathy, pancreatitis, and insulin resistance. In effect, the balance between these two axes may determine the prognosis. The already strained ACE-2-Ang-(1–7)-Mas in metabolic disorders is further stressed due to the use of the ACE-2 by the virus for entry, which affects the prognosis in terms of respiratory compromise. Further evidence needs to be gathered on whether modulation of the renin angiotensin system would be advantageous due to upregulation of Mas activation or harmful due to the concomitant ACE-2 receptor upregulation in the acute management of COVID-19.

ABBREVIATIONS

ACE Angiotensin converting enzyme
Ang Angiotensin
ARDS Acute respiratory distress syndrome
AT1R Angiotensin 1 receptor
RAS Renin angiotensin system
SARS Severe acute respiratory syndrome
Introduction

Coronaviruses (CoV) are a large family of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV). The SARS-CoV-2, has caused a rapidly spreading outbreak (COVID-19) with over 300,000 infected cases and more than 13,000 deaths globally [1–8]. The SARS-CoV-2, a positive strand RNA virus, has been seen to infect humans through the angiotensin converting enzyme-2 (ACE-2) receptor [9].

In COVID-19 infections, emerging evidence demonstrates that older individuals and people with underlying metabolic conditions of diabetes mellitus, hypertension, and hyperlipidemia are at higher risk of morbidity and mortality [1–8].

In individuals with hypertension, diabetes, and other cardiovascular disorders with vascular complications, the renin angiotensin system (RAS) is known to be elicited with an increase in ACE activity and a downregulation of ACE-2. Modulation of this system by ACE-inhibitors or AT1-Receptor blockers is now considered as the first-line therapy as well as prevention and management of vascular complications.

In this regard, the questions arise if (i) differences in ACE-2 may explain the exacerbated course of disease in patients with metabolic diseases and (ii) if ACE modulation in COVID-19 patients is neutral, beneficial, or harmful. The latter question may have immediate therapeutic consequences for millions of patients. Moreover, ACE-2-based therapy has been proposed as a potential therapeutic approach in COVID-19 pneumonia [10].

The ACE-2 enzyme and infection with SARS-CoV

The angiotensin converting enzyme 2 (ACE-2), a single pass type 1 membrane monooxidasepeptidase, discovered 2 decades ago [11], consists of an N-terminal peptidase domain and C-terminal collectrin like domain [9]. It is the peptidase domain that is responsible for the main functions of the renin angiotensin system (RAS) [9]. The ACE-2 shares 40% homology with the N-terminal catalytic domain of ACE, and a hydrophobic region near the C-terminus likely to serve as a membrane anchor [9, 11]. The ACE-2 protein is encoded by the ACE-2 gene located on chromosome Xp22. These ACE-2 proteins are more abundantly expressed on the apical surface of the well-differentiated and mostly ciliated airway epithelium of the lungs (alveolar Type-2 cells), and enterocytes of the small intestine [12]. Furthermore, ACE-2 protein is expressed in arterial and venous endothelial cells and arterial smooth muscle cells, in the heart, kidneys, adrenal glands, pancreas, skeletal muscle, and adipose tissues [11].

The coronavirus SARS-CoV-2, a single stranded RNA virus, has been seen to infect humans through their envelope spike glycoprotein (S-protein), which is responsible for CoV cell entry and host-to-host transmission. During viral infection, this S-protein cleaves into S1 and S2 [9]. The FURIN cleavage site in the SARS-CoV-25 protein may provide a priming mechanism [13]. The ectodomain S1 binds to the peptidase domain of the ACE-2 enzyme, while the S2 is cleaved further by the host cell serine protease TMPRSS2 [14] resulting in membrane fusion. Both these steps are essential for the viral entry into the cells.

An in vivo study shows that the infection of human airway epithelia by SARS coronavirus correlated with the state of cell differentiation and ACE-2 expression and localization [15]. The infection tends to occur more readily through well differentiated ciliated cells with higher ACE-2 expression [15]. It has been observed that ACE-2 membrane expression and plasma levels are reduced after infection with SARS coronavirus [16]. Further, SARS CoV spike protein has been found to reduce ACE-2 expression and to augment pulmonary injury [16]. However, treatment with blockers of the renin-angiotensin system reduced the pulmonary injury by activating the ACE-2-Ang-(1–7)-Mas axis.

The Renin-angiotensin system

In the classical endocrine model of the RAS, renin cleaves its substrate, angiotensinogen (AGT), to produce the inactive peptide, angiotensin I (Ang I), which is then converted to angiotensin II (Ang II) by endothelial angiotensin-converting enzyme (ACE). The catalytic activity of ACE to activate Ang II occurs most extensively in the lung. Ang II mediates vasoconstriction as well as aldosterone release from the adrenal gland, resulting in sodium retention and an increase in blood pressure through the angiotensin 1 receptor (AT1R).

However, recent evidence suggests that RAS also includes local systems with autocrine (cell-to-same cell) and paracrine (cell-to-different cell) effects in addition to the classical circulating RAS with its well-known classical endocrine effects. In particular, Ang II generation at the tissue level by the tissue specific RAS appears to have physiologic effects that are as important as circulating/systemic Ang II and, under some circumstances, more important than circulating Ang II. Therefore, the RAS system is not only involved in controlling blood volume and blood pressure but with the tissue specific local systems it directs tissue remodeling, endothelial dysfunction, and fibrosis [17–20].

Simplistically, it can be seen as a dual function system, which acts through two apparently opposite arms: the one responsible for the main actions of this system is constituted by the ACE Ang II-AT1 receptor axis and the other, a counterregulatory arm, is formed by the ACE-2-Ang-(1–7)-Mas axis. The catalytic activity of ACE results in increased Ang II levels and increased catabolism of Ang-(1–7) whereas the catalytic activity of ACE-2 is predominantly on Ang I and Ang II and leads to formation of Ang-(1–7). Ang-(1–7) acts on the G-protein coupled receptor Mas and is known to have counterregulatory actions on Ang II resulting in vasodilatation and growth inhibitory effects [17–20] (See Fig. 1).

The Renin-angiotensin system in metabolic disorders

A common mechanism by which diabetes, hypertension, and other metabolic disorders cause vascular complications is endothelial dysfunction, inflammation, and atherosclerosis. In these disorders, the ACE-Ang II-AT1 receptor axis is activated with a downregulation of ACE-2-Ang-(1–7)-Mas axis [19–21], which leads to increased local and circulatory Ang II with a decrease in Ang-(1–7) and subsequent increased oxidative stress, activation of the endothelium, smooth muscle cell migration, growth, proliferation and thrombosis [19–21]. Deficiency in ACE-2 leads to increased formation of atherosclerotic plaques and blocking of ACE-2 results in prevention of atherosclerosis [20] (Fig. 1). ACE-2 is present in multiple
organs and has various functions. In the heart, ACE-2 prevents progressive cardiac fibrosis associated with aging and/or cardiac pressure overload and is beneficial in heart failure [19, 20]. In most forms of chronic kidney disease including diabetic and hypertensive nephropathy, expression of ACE-2 has been reported to be reduced in kidney tubules with an increased expression in the glomerula [20]. This imbalance has been postulated as a potential cause of diabetic nephropathy.

In the adrenal glands, the local secretory RAS stimulates aldosterone production and serves as an amplification system for circulating Ang II. Importantly the regulation of the secretory adrenal RAS is independent of the circulatory RAS and the activity of the adrenal RAS correlates with aldosterone production and regulation of potassium serum concentrations [22]. Severe diabetes has been associated with hyporeninaemic hyperactivity of the adrenal gland [23].

In the pancreas, the ACE-2-Ang-(1–7)-Mas axis has been described to protect the function of insulin-producing beta cells by improving the function of islet microvascular endothelial cells. Further, activation of endothelial nitric oxide synthase and NO signaling pathways via the ACE-2-Ang-(1–7)-Mas axis may have anti-inflammatory beneficial effects in pancreatitis [23, 24]. In the skeletal muscle, the ACE-2-Ang-(1–7)-Mas axis has been described to decrease insulin resistance [25]. ACE-2 may exert potential anti-obesity effects via stimulating brown adipose tissue formation and induction of browning in white adipose tissue [26].

A study done in the survivors of the SARS infections, the organ involvement correlated with the organ expression of ACE-2 has been reported to be reduced in kidney tubules with an increased expression in the glomerula [20]. This imbalance has been postulated as a potential cause of diabetic nephropathy.

In the respiratory system, activation of ACE leads to a proinflammatory, pro-fibrotic, pro-hyperresponsiveness response in the respiratory system, whereas ACE-2-Ang-(1–7)-Mas induces a protective mechanism of anti-inflammatory, anti-fibrotic and anti-hyperresponsiveness. A lower ACE-2 will put these individuals at higher risk of respiratory distress. In hyperension, diabetes, and CVD, the ACE related pathway is activated with downregulation of the ACE-2 pathway. These results in the multi-organ complications seen in metabolic diseases with endothelial dysfunction promoting atherosclerosis, increased cardiac fibrosis and LV remodeling, diabetic nephropathy, hyperactivity of adrenal gland, and it decreases insulin release and increases insulin resistance.

Infection with COVID-19 may exacerbate the ACE-2 deficiency in these patients in all organs and maybe responsible for the multiorgan failure.

The renin angiotensin system and the respiratory system

The respiratory system is a major site of ACE-activity and source of systemic Ang II synthesis. Locally produced Ang II may trigger increasing vascular permeability facilitating pulmonary edema. The ACE-2-Ang-(1–7)-Mas axis that is highly expressed in the lungs, may potentially induce pulmonary vasoconstriction in response to hypoxia, which is an important process in preventing shunting in patients with pneumonia or lung injury [19]. In acute respiratory distress syndrome (ARDS) mouse models, ACE-2 knockout mice displayed more severe ARDS symptoms compared with wildtype mice, while overexpression of ACE-2 appeared to be protective [19].

Thus, stimulation of the ACE-2-Ang-(1–7)-Mas axis may have anti-inflammatory and antifibrotic effects in the pulmonary system, and these actions could potentially be favorable in the recovery of patients with pulmonary inflammation. It is possible that an ACE/ACE-2 imbalance is one of the potential mechanisms explaining why patients with cardio-metabolic problems are at higher risk for respiratory failure [20].

In an animal model study, it has been reported that infusion of SARS CoV spike protein reduced ACE-2 expression and augmented pulmonary injury. However, treatment with blockers of the renin-angiotensin system reduced the pulmonary injury by activating the ACE-2-Ang-(1–7)-Mas axis [16]. Therefore, it may be assumed that the inherent downregulation of the ACE-2-Ang-(1–7)-Mas axis (as seen in metabolic conditions) is exacerbated in the COVID-19 state because (i) the virus uses the peptidase domain of the enzyme for entry into the cells and (ii) there is a decrease in ACE-2 with an increase in ACE [9].

Pathological features of COVID-19 infection

In COVID-19 infections, chest radiography typically shows patchy or diffuse asymmetric airspace opacities bilaterally more in the peripheral zones and in later stages ground glass opacities. CT Scan typically shows bilateral involvement, which is different from SARS and MERS which starts unilaterally. Moreover, it shows bilateral multifocal ground-glass opacities with a peripheral lung involvement in milder cases with more widespread consolidation in patients who go to the intensive care unit. Pleural effusion, cavitation, pulmonary nodules, and lymphadenopathy have not been reported in patients with COVID-19. An important clinical observation of COVID-19 infection in China was that some individuals with early infection exhibited no symptoms. Interestingly, they even had negative swab results (due to absence of infection in the upper respiratory tract) and normal chest X-rays, however, showed significant changes in the CT scan of the lungs [28].

Histopathological examination of early COVID-19 infected lungs, which were serendipitously detected in specimens operated for lung cancer, revealed exudative and proliferative phases of acute lung injury, edema and congestion of alveolar septa with inflammatory infiltrates (macrophages), type II pneumocytes hyperplasia with viral inclusions, organization of inflammatory exudates and interstitial fibrosis [29]. These pathological findings corroborated with the CT findings of patchy ground glass opacities. Intriguingly, these pathological findings of inflammatory and fibrotic changes are known to be exacerbated in animal models of ACE-2 knockout mice [19].

Histopathological examination from autopsies of individuals who have succumbed from COVID-19 infections showed degenerated and necrotized cells in the myocardium and blood vessels with interstitial inflammatory infiltrates and focal necrosis. In the kidneys, proteinaceous exudates in the bowman’s capsule surrounding the glomerulus, degeneration and shedding of the renal tubular epithelial cells and hyaline casts, microthrombi and fibrotic foci have been reported [30, 31]. Some of these changes of myocardial fibrosis and renal changes are already seen in the chronic phase of metabolic disorders due to ACE-2 downregulation and these may get exacerbated [19, 20].

Observations from clinical presentation and course of COVID-19 infections

The vast majority of COVID-19 patients have mild symptoms. However, a significant percentage is critically ill with admission to an intensive care unit (ICU, approx. 10 %) with 4 % case fatality rate globally [John Hopkins Coronavirus Resource Center: https://coronavirus.jhu.edu/]. Mortality is higher in patients with underlying hypertension, type-2 diabetes mellitus, or cardiovascular disease [1–3]. To address this further, we performed a cumulative analysis of published data from China and South Korea, which showed that a disproportionately higher percentage of patients with a higher morbidity (requiring ICU care, ARDS) had hypertension (28 %), type-2 diabetes (17 %), CVD (8 %) as an underlying disease. Moreover, patients admitted to the ICU had a higher systolic BP when compared to asymptomatic cases [4–7] and in a recent series reported none of the nonsurvivors had hypotension [3] (Table 1).

A limitation of the data summarized in Table 1 is the possibility that some cases were reported twice in different series. Interestingly, however, in a recent case report on a mild case extensively followed in Australia, a low level of the monocyte chemoattractant protein-1 (MCP-1), a key biomarker of type-2 diabetes related vascular complications, was found to be associated with minor disease pathology [32]. In a recent preprint study, hypokalemia due to renal potassium wasting has been reported with 93 % of the critically ill patients having hypokalemia independent of gastrointestinal symptoms [33].

The presence of higher expression of ACE-2 receptors or its up-regulation although initially thought to be a risk factor for infection is unlikely the case. In a recent preprint study, wherein they integrated public genomics, epigenomics, and transcriptomics data, they found that the expression of ACE-2 is relatively high in Asian females and young people while it is lower in males, and further decreases with age and with the progression of type-2 diabetes [34]. This contradicts the hypothesis that higher expression is directly related to increased susceptibility as we know that SARS-CoV-2 infections are more common in males and in elderly patients with metabolic disorders.

Taken together, these observations suggest that patients with underlying type-2 diabetes, hypertension and/or cardiovascular diseases are at higher risk for requiring critical care and ventilation. There is evidence that although they are critically ill, they tend to demonstrate normotension to hypertension and hypokalemia, which are classical features of an activated ACE related RAS with a downregulation of the ACE-2 pathway. As per current data, it does not appear that higher ACE-2 expression is associated with an immediate predilection as individuals at risk will tend to have lower ACE-2 expression.

Therapeutic modulations

Since ACE-2 is the entry receptor for cellular infection by SARS-CoV-2, blocking entry using ACE-2-related therapy could be feasible to prevent the spreading of infection in the lungs and the whole body. Convalescent sera containing neutralizing antibodies against SARS-S protein offer protection against SARS-CoV-2 infection [35]. Therefore, it was suggested to use recombinant human ACE-2 protein to saturate the viral S-protein and thus prevent cellular entry of SARS-CoV-2 [10]. This is especially of interest, since ACE-2 has already been de-
Another crucial step in cellular infection involves the serine protease TMPRSS2 that cleaves the viral S-protein. The TMPRSS2 inhibitor camostat mesylate has been approved in Japan for pancreatic inflammation and has been shown to prevent cellular infection by SARS-Cov-2 [14]. Consequently, it could prove being useful in Covid-19.

Both ACE inhibitors and AT₁ receptor blockers have been used in diabetes and cardiovascular disorders to modulate this system in preference for vasodilation and maintenance of endothelial integrity. Furthermore, in the diabetic kidney, reduced ACE-2 protein expression could be prevented by ACE inhibitor therapy suggestive of an upregulation with ACE inhibitor [19]. It is worthwhile men-

Table 1 Mortality (death) and morbidity (ICU stay) associated with cardiometabolic disease based on reported cases in the literature.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Country</th>
<th>Total (n)</th>
<th>Hypertension</th>
<th>Diabetes</th>
<th>CVD</th>
<th>BP</th>
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<tr>
<td>4. Huang C et al. Lancet 2020; 395 (10223): 497–506 [4]</td>
<td>China</td>
<td>Total: 41 Non-ICU: 28 ICU:13</td>
<td>Non-ICU: 4 (14.3 %) vs. ICU: 2 (15.3 %); p = 0.93</td>
<td>Non-ICU: 7 (25 %) vs. ICU: 1 (7.7 %); p = 0.16</td>
<td>Non-ICU: 3 (10.7 %) vs. ICU: 3 (23 %); p = 0.32</td>
<td>Sys BP: Non-ICU: 145 (123–167) vs. ICU: 122 (118–130); p = 0.018</td>
</tr>
<tr>
<td>5. Wang D et al. JAMA 2020; doi: 10.1001/jama.2020.1585 [5]</td>
<td>China</td>
<td>Total: 138 Non-ICU: 102 ICU: 36</td>
<td>Non-ICU: 22 (21.6 %) vs. ICU: 21 (58 %); p &lt; 0.001</td>
<td>Non-ICU: 6 (5.9 %) vs. ICU: 8 (22.2 %); p = 0.009</td>
<td>Non-ICU: 11 (10.8 %) vs. ICU: 9 (25 %); p = 0.001</td>
<td>MAP: Non-ICU: 90 (85–98), vs. ICU: 91 (78–96); p = 0.33</td>
</tr>
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<td>6. Guan W J et al. NEJM 2020; doi: 10.1056/NEJMoa2002032 [6]</td>
<td>China</td>
<td>Total: 1099 Non-Severe: 926 Severe: 173</td>
<td>Non-Severe:124 (13.4 %) vs. Severe: 41 (23.7 %); p &lt; 0.001</td>
<td>Non-Severe: 53 (5.7 %) vs. Severe: 28 (16.2 %); p &lt; 0.001</td>
<td>Non-Severe: 17 (1.8 %) vs. Severe: 10 (5.8 %); p &lt; 0.001</td>
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<tr>
<td>7. Wu C et al. JAMA Intern Med 2020; doi: 10.1001/jamainternmed.2020.0994 [7]</td>
<td>China</td>
<td>Total : 201 Non ARDS: 117 ARDS : 84</td>
<td>Non-ARDS: 16 (13.7 %) vs. ARDS: 23 (27.4 %); p = 0.02</td>
<td>Non-ARDS: 6 (5.1 %) vs. ARDS: 16 (19 %); p = 0.002</td>
<td>Non-ARDS: 3 (2.6) vs. ARDS: 5 (6 %); p = 0.40</td>
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<tr>
<td>Consolidated chi-square test for patients reported in references 4–7 above [4–7]</td>
<td>China</td>
<td>Total: 1479 Non-severe: 1173 Severe: 306</td>
<td>Non-severe: 166 (14 %) vs. Severe: 87 (28.4 %); p &lt; 0.0001</td>
<td>Non-severe: 72 (6 %) vs. Severe: 53 (17.3 %); p &lt; 0.0001</td>
<td>Non-severe: 34 (2.9 %) vs. Severe: 27 (8.8 %); p &lt; 0.0001</td>
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ICU: Patients requiring Intensive Care Unit; Non-ICU: Patients not requiring intensive care unit; BP: Blood Pressure; Sys BP: Systolic blood pressure; MAP: Mean arterial pressure; ARDS: Patients with acute respiratory distress syndrome; Non-ARDS: Patients without acute respiratory distress syndrome. * p < 0.001.
tioning that ACE-2 receptor upregulation due to angiotensin receptor blockers that are commonly used in hypertension could be considered to be risk factor for increased transmission of SARS-CoV-2. However, it has been seen that the AT1 receptors tend to associate with ACE-2 in these cases, which prevent the internalization of the ACE-2 receptor and hence may not be amenable to viral entry [40]. Moreover, as mentioned earlier, higher expression of ACE-2 was not associated with higher susceptibility to infection with SARS-CoV.

Clinical implications

According to the scientific data detailed above, it is tempting to speculate that blocking the ACE-Ang II-AT1 receptor axis with AT1 blockers commonly used as antihypertensives may tilt the balance in favor of the ACE-2-Ang-(1–7)-Mas axis, which may help to accelerate respiratory recovery in patients suffering from COVID-19 [41]. However, it is intriguing that the patients most likely to be treated with ACE-I or AT1 blockers are the ones with the poorest prognosis in COVID-19 [1–7]. Moreover, ACE-2 upregulation concomitant with RAS inhibitions may ease viral infection. Consequently, it is also possible, that RAS blockade may have a detrimental effect in COVID-19. Currently, there is no clinical data to support one or the other direction. Thus, the Heart Failure Society of America, the American College of Cardiology, and the American Heart Association have all issued statement to suggest that there is no definitive evidence of harm or benefit with the use of ACE-inhibitors or AT1 receptor blockers and that patients should continue to take their medications as usual [42]. It is of the utmost priority to evaluate the growing stock of clinical data from China and worldwide to determine if RAS inhibitors are beneficial or deleterious in COVID-19. Only then, we can know how to counsel our patients taking RAS inhibitors. In fact some researchers are planning a clinical trial to study losartan in COVID-19 patients [43].

Conclusion

Individuals with underlying hypertension, type 2 diabetes, or cardiovascular disease are at higher risk for respiratory failure and mortality in COVID-19. One of possible mechanisms for this predilection may be the imbalance in the ACE pathways and therapeutics targeting viral entry through the ACE-2 receptor or the ACE-Ang II-AT1 receptor axis as well as stimulating the ACE-2-Ang-(1–7)-Mas axis may be beneficial in these individuals. Further evidence needs to be gathered on whether modulation of the renin angiotensin system would be advantageous due to upregulation of Mas activation or harmful due to the concomitant ACE-2 receptor upregulation in the acute management of COVID-19.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References


[13] Lukassen S, Chua RL, Trefzer T et al. SARS-CoV-2 receptor ACE2 and TMPRSS2 are predominantly expressed in a transient secretory cell type in subsegmental bronchial branches (preprint); bioRxiv. 2020; doi:10.1101/2020.03.13.991455


[34] Chen J, Liang Q, Xia X et al. Individual variation of the SARS-CoV2 receptor ACE2 gene expression and regulation. Preprints 2020; 2020030191


[38] Wosten-van Asperen RM, Lutter R, Specht PA et al. Acute respiratory distress syndrome leads to reduced ratio of ACE/ACE-2 activities and is prevented by Ang-(1-7) or an Ang II receptor antagonists. J Pathol 2011; 225: 618–627


[40] Deshotels MR, Xia H, Sriramula S et al. Angiotensin II mediates angiotensin converting enzyme type 2 internalization and degradation through an angiotensin II receptor type 1 receptor-dependent mechanism. Hypertension 2014; 64: 1368–1375

