

# Angiotensin II, RAS Activation, and RAS Blockers in COVID-19: Unambiguous Evidence

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## Keywords

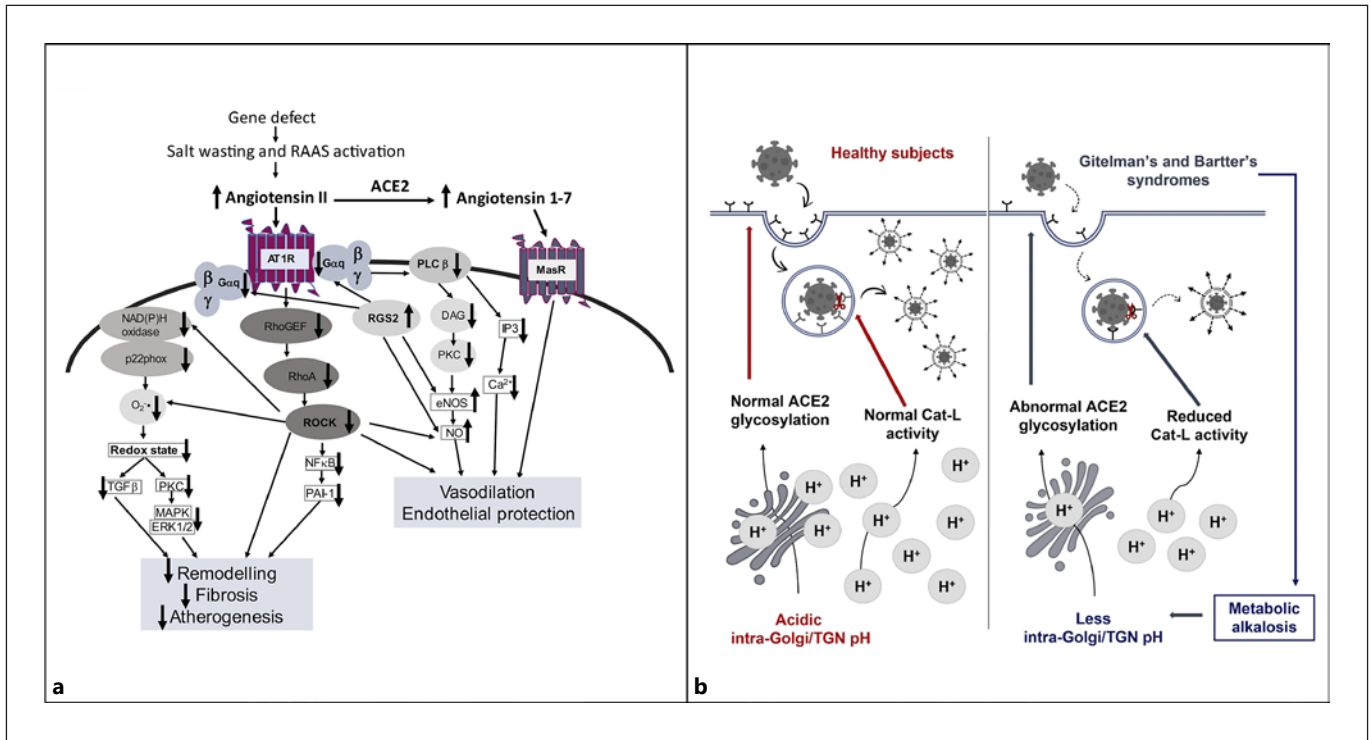
Angiotensin II · RAS blockers · COVID-19 · Gitelman's syndrome · Bartter's syndrome

The renin-angiotensin system (RAS) during COVID-19 pandemic has captured the interest as the severe coronavirus 2 (SARS-CoV-2) causing COVID-19 uses angiotensin-converting enzyme type 2 (ACE2) as an entry point into the cell [1]. ACE2 is one of the key regulators of the RAS being a part of the protective counter-regulatory ACE2-Ang 1-7-Mas receptor (MasR) axis, which opposes the classical ACE-angiotensin II (Ang II)-Ang II type 1 receptor (AT1R) regulatory axis of the RAS [2].

In patients with conditions featuring an activated RAS, as cardiovascular and renal patients that use RAS blockers, such as ACEIs or Ang II type 1 receptor blockers (ARBs), these drugs were suggested to be detrimental in COVID-19 patients as they increase ACE2 expression potentially facilitating the SARS-CoV-2 entry into the cell [3]. This, however, has been widely criticized [4]. It is known, in fact, that SARS-CoV-2 causes ACE2 downregulation impairing the ACE2-mediated Ang II conversion

into Ang 1-7 with the loss of its vasodilatory, anti-inflammatory, antioxidant, and anti-atherosclerotic properties and of their protective impact on COVID-19 morbidity and mortality [5]. RAS blockers, life/organ-saving drugs for the above mentioned patients with activated RAS, have been shown to be beneficial in conditions of RAS activation due not only to their blunting/blocking of Ang II signaling via AT1R-mediated vasoconstriction and inflammation [6] but also via induction of the counter-regulatory arm of the RAS system, which includes ACE2, Ang 1-7, and Mas receptor (MasR) [4].

Pretreatment with captopril or candesartan, in fact, prevents SARS-CoV-2 spike protein internalization into human type II pneumocytes as well as spike protein-induced proinflammatory cytokine response, and 3 weeks of treatment with captopril or candesartan upregulates ACE2 and MasR in the rat's lung [6]. In addition, a demonstration that ACEI and ARBs are protective in SARS-CoV-2 infection comes by the report in the lung tissue of aged rats and rats with metabolic syndrome that RAS dysregulation (reduced expression of ACE2, Ang II-type 2 receptors [AT2R], and MasR) was reversed by captopril or candesartan [6], confirming that ACEI and ARBs contribute to decreasing the viral entry and reducing the pro-



**Fig. 1.** **a** Endogenous downregulation of Ang II signaling via AT1R downstream the AT1R activation in GS/BS patients which is the mirror image of hypertension and represents an endogenous antagonism of Ang II signaling via AT1R, mimicking the effects of RAS blockers. **b** Possible mechanism of altered ACE2 glycosylation and reduced Cat-L activity in GS/BS causing reduced SARS-CoV-2 entry into the cell and reduced proteolytic cleavage of SARS-CoV-2 into the cell.

inflammatory cytokine release despite the increased expression of ACE2 [6]. Finally, genetic deletion of ACE2 worsens experimental acute respiratory distress syndrome in knockout mice models, while Ang 1-7 and ACEI or ARB administration attenuate the inflammatory response, markedly decrease lung injury scores and improve lung function [7].

Recently, another important piece of evidence of the beneficial role of RAS blockers in COVID-19 patients with activated RAS has been provided by an in vitro study on human epithelial bronchial cells via an elegant approach of infectivity and spike-mediated cell-cell fusion assay, mimicking the viral attachment and the entry steps of the SARS-CoV-2 [8]. This study investigating how Ang II and RAS blockers affected ACE2 expression and SARS-CoV-2 infectivity showed that Ang II, via AT1R, increases mRNA and protein level of ACE2, resulting in enhanced SARS-CoV-2 cell entry, effects which were abolished by irbesartan, an ARB. These results provide a mechanistic rationale for both the protective action of

ARBs and the lack of worse outcomes in high-risk COVID-19 patients that use RAS blockers [8].

In vivo human data that may address the beneficial role of RAS blockers in COVID-19 and its complications may be provided by patients with Gitelman's and Bartter's syndromes (GS/BS). These rare genetic tubulopathies, which we have deeply investigated for their biochemical and hormonal characteristics, include high Ang II levels and RAS activation yet normo-hypotension, hypokalemia, and metabolic alkalosis; downregulation of Ang II signaling via AT1R; increased ACE2 and Ang 1-7 levels; activation of Ang II signaling via AT2R; protection from cardiovascular and renal remodeling [9, 10] and represent a model of endogenous antagonism of Ang II signaling via AT1R [9, 10] (Fig. 1a).

In addition to the demonstration of the endogenous antagonism of Ang II signaling via AT1R in the face of activated RAS and high Ang II levels, which mimics the effect of RAS blockers and of activation of the counter-regulatory arm of RAS we have provided in these patients

[9], we have recently shown in GS/BS patients a reduced ACE2 glycosylation and cathepsin (Cat)-L activity [11], this latter is a protease involved in the proteolytic cleavage of SARS-CoV-2 in the cell [1, 12]. Reduced ACE2 glycosylation and Cat-L activity occur in these patients likely via the effect of their metabolic alkalosis-induced changes of pH in the host intracellular organelles' environment whose acidic pH is necessary both for ACE2 glycosylation and Cat-L activity (Fig. 1b). The reduction of the acidic pH/alkalinization of intracellular organelles' environment may be responsible for both of an altered capacity of SARS-CoV-2 to bind its ACE2 receptor and therefore its entry in the cell and of proteolytic cleavage of SARS-CoV-2 into the cell by Cat-L [11, 13]. All these findings seem to confer protection against the SARS-CoV-2 severe form of infection [11], which has been proven by 3 different surveys we have assessed in our cohort of GS/BS patients.

A first survey on our cohort of 128 GS/BS patients living in the Northern Italy hotspots of COVID-19 pandemic in early 2020 during the first wave of COVID-19 showed a significantly reduced susceptibility to COVID-19 infection in these patients compared to the prevalence of COVID-19 in the general northern Italian population, as none of them had been affected or had symptoms related to the SARS-CoV-2 infection [14]. A second survey on the same cohort 1 year later during the third wave of COVID-19 in April 2021 found that only 8 patients out of 128 tested positive for COVID-19, of which 4 were asymptomatic and 4 had very mild symptoms [11].

A third survey was assessed on the same cohort in December 2021–January 2022 during the peak of the fourth wave of COVID-19 induced by the Omicron variant. The results of this latter survey confirm those of the previous surveys, showing that 14 patients out of 128 tested positive, 9 asymptomatic, and 5 with mild symptoms, 4 of this latter had refused the vaccination [11].

The impact of the high number of vaccinated patients may have contributed to reduce the severity of the disease. However, the results of our third survey confirm the GS/BS patients' resistance to SARS-CoV-2 infection and COVID-19 severity and further support the mechanistic explanation for their resistance based both on the endogenous antagonism of Ang II signaling via AT1R [9] and on changes of pH in the host intracellular organelles' environment-induced altered ACE2 glycosylation and reduced Cat-L activity [11, 13].

We believe that the results of these studies in GS/BS patients [11, 14] joined to those we have provided in these patients to better define the human RAS system [9, 10]

strongly support data in a human model characterized by an endogenous antagonism of Ang II signaling via AT1R [9], the results of the above mentioned study by Caputo and coworkers [8]. This study, in fact, using an in vitro approach demonstrated that an activated RAS increased the susceptibility to SARS-CoV-2 infection and that RAS blockers abolished the virus entry in the cell [8], supporting, indirectly but unambiguously, the protective effect of these drugs in patients such as cardiovascular and renal disease patients treated with RAS blockers, which therefore should not be discontinued. Finally, the protection from COVID-19 in GS/BS patients proven also with the demonstration of the reduction of Cat-L activity [11] leading to an altered proteolytic cleavage of SARS-CoV-2 into the cell [11, 13, 15] has the same mechanistic rationale on which is based the effect of Paxlovid, the new available drug to fight COVID-19 [16, 17].

### Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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### Author Contributions

Conceptualization and writing – original draft preparation: Lorenzo A. Calò; bibliographic search: Lucia Federica Stefanelli and Federico Nalesso. All the authors have read and agreed to the published version of the manuscript.

### References

- 1 Wang Q, Zhang Y, Wu L, Niu S, Song C, Zhang Z, et al. Structural and functional basis of SARS-CoV-2 entry by using human ACE2. *Cell*. 2020;181(4):894–904.e9.
- 2 Paz Ocaranza M, Riquelme JA, Garcí 'a L, Jalil JE, Chiong M, Santos RAS, et al. Counter-regulatory renin-angiotensin system in cardiovascular disease. *Nat Rev Cardiol*. 2020;17(2):116–29.
- 3 Fang L, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? *Lancet Respir Med*. 2020;8(4):e21.
- 4 Simko F, Baka T. Angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers: potential allies in the COVID-19 pandemic instead of a threat? *Clin Sci*. 2021;135(8):1009–14.

- 5 Baral R, Tsampasian V, Debski M, Moran B, Garg P, Clark A, et al. Association between renin-angiotensin-aldosterone system inhibitors and clinical outcomes in patients with COVID-19: a systematic review and meta-analysis. *JAMA Netw Open*. 2021;4(3):e213594.
- 6 Pedrosa MA, Valenzuela R, Garrido-Gil P, Labandeira CM, Navarro G, Franco R, et al. Experimental data using candesartan and captopril indicate no double-edged sword effect in COVID-19. *Clin Sci*. 2021;135(3):465–81.
- 7 Wosten-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(4):618–27.
- 8 Caputo I, Caroccia B, Frasson I, Poggio E, Zamberlan S, Morpurgo M, et al. Angiotensin II promotes SARS-CoV-2 Infection via up-regulation of ACE2 in human bronchial cells. *Int J Mol Sci*. 2022;23(9):5125.
- 9 Calò LA, Davis PA, Rossi GP. Understanding the mechanisms of angiotensin II signaling involved in hypertension and its long-term sequelae: insights from Bartter's and Gitelman's syndromes, human models of endogenous angiotensin II signaling antagonism. *J Hypertens*. 2014;32(11):2109–19; discussion 2119.
- 10 Calò LA, Maiolino G. Mechanistic approach to the pathophysiology of target organ damage in hypertension from studies in a human model with characteristics opposite to hypertension: Bartter's and Gitelman's syndromes. *J Endocrinol Invest*. 2015;38(7):711–6.
- 11 Bertoldi G, Ravarotto V, Sgarabotto L, Davis PA, Gobbi L, Calò LA. Impaired ACE2 glycosylation and protease activity lowers COVID-19 susceptibility in Gitelman's and Bartter's syndromes. *J Intern Med*. 2022;291(4):522–4.
- 12 Liu C-L, Guo J, Zhang X, Sukhova GK, Libby P, Shi G-P. Cysteine protease cathepsins in cardiovascular disease: from basic research to clinical trials. *Nat Rev Cardiol*. 2018;15(6):351–70.
- 13 Calò LA, Davis PA. Far di Necessità Virtù, using rare tubulopathies, Gitelman's and Bartter's syndromes, to inform the fight against COVID-19. *J Nephrol*. 2021;34(2):281–3.
- 14 Calò LA, Rigato M, Sgarabotto L, Giansello L, Bertoldi G, Ravarotto V, et al. ACE2 and SARS-CoV-2 infection risk: insights from patients with two rare genetic tubulopathies, Gitelman's and Bartter's syndromes. *Front Med*. 2021;8:647319.
- 15 Sgarabotto L, Giansello L, Bertoldi G, Nalesso F, Stefanelli LF, Calò LA. Rare genetic tubulopathies Gitelman's and Bartter's syndromes and their naturally occurring protection from COVID-19. *Minerva Med*. 2022 Apr 6. Epub ahead of print.
- 16 Ledford H. COVID antiviral pills: what scientists still want to know. *Nature*. 2021;599(7885):358–9.
- 17 Resnick SJ, Iketani S, Hong SJ, Zask A, Liu H, Kim S, et al. Inhibitors of coronavirus 3CL proteases protect cells from protease-mediated cytotoxicity. *J Virol*. 2021;95(14):e0237420.