

Perspective

ACE2, Metformin, and COVID-19

Atul Malhotra,^{1,2,*} Mark Hepokoski,^{1,2,5} Karen C. McCowen,^{1,3} and John Y-J Shyy^{1,4,*}

SUMMARY

COVID-19 is becoming a leading cause of mortality throughout the world, and few effective therapies are currently available. Angiotensin converting enzyme 2 (ACE2) is essential to COVID-19 pathogenesis, as the binding of SARS-CoV-2 spike protein (S protein) is required for viral entry and development of COVID-19. ACE2 regulates the protective arm of the renin-angiotensin-aldosterone system (RAAS) that endows anti-hypertensive and anti-inflammatory effects in the cardiovascular and pulmonary systems. Preclinical data suggest ACE2 might be downregulated after SARS-CoV-2 binding, and treatments that increase ACE2 may prevent cardiopulmonary injury. Development, testing, and mass production of novel ACE2 therapies may take years, whereas more effective treatments for COVID-19 are needed urgently. Metformin is a widely available anti-diabetic agent that has an excellent safety profile, and clinical and preclinical data suggest metformin may offer cardiopulmonary protection in COVID-19 via enhanced ACE2 expression.

INTRODUCTION

With the COVID-19 pandemic a threat to human health worldwide, there is an urgent need to identify treatments that are safe and available immediately. The virus responsible for COVID-19, SARS-CoV-2, has a spike protein (S protein) binding domain that is nearly identical in structure to the virus responsible for prior outbreaks of severe acute respiratory syndrome (SARS), SARS-CoV-1 (Lan et al., 2020). However, the binding affinity of SARS-CoV-2 for the angiotensin converting enzyme 2 (ACE2) receptor appears to be 10 times stronger, which may partially explain its rapid spread throughout the world (Wrapp et al., 2020). ACE2 binding in pneumocytes and enterocytes is required for viral entry, yet paradoxically, treatments that increase ACE2 may be beneficial in mitigating the complications of COVID-19. In the renin-angiotensin-aldosterone system (RAAS), Angiotensin converting enzyme 2 (ACE2)-Ang 1-7-Mas acts as the protective arm to counteract the deleterious arm of ACE1-Ang II to reduce systemic and pulmonary hypertension and activate anti-inflammatory pathways after tissue injury (Santos et al., 2018). Interestingly, SARS-CoV-1 was found to decrease ACE2 expression after binding, and low levels of ACE2 have been implicated in various cardiovascular impairments and acute respiratory distress syndrome (ARDS) (Reddy et al., 2019). Recently, treatment with human recombinant ACE2 (hrACE2) showed positive results in a human organoid model of SARS-CoV-2 (Monteil et al., 2020), but safety and efficacy of hrACE2 still need to be determined in human studies and timely production might not meet the need for patients. Here, we discuss the rationale for metformin as a safe and currently available therapy that is known to increase ACE2 and may offer cardiopulmonary benefit in patients suffering from COVID-19.

Data collected from cohorts in Wuhan, China, indicate high comorbidity of hypertension, diabetes mellitus (DM), and cardiovascular diseases among patients with COVID-19 (Chen et al., 2020). The prevalence of hypertension in COVID-19 draws tremendous attention to using RAAS inhibitors, i.e., ACE inhibitors (ACEI) and angiotensin receptor blockers (ARBs), in treating COVID-19 symptoms and the underlying diseases (Vaduganathan et al., 2020). Ample preclinical and clinical studies have been conducted to investigate the effects of ACEI and ARBs on the expression and activity on ACE2 (Ferrario et al., 2005). However, these studies have generated conflicting results and the effects of these drugs on ACE2 in humans remain inconclusive (Ramchand et al., 2018). Nonetheless, consensus is emerging that potential benefits override the potential harms of RAAS inhibitors in COVID-19 (Vaduganathan et al., 2020), possibly via ACE2 augmentation.

Despite the American Heart Association statement advocating “not to add or remove any RAAS-related treatments, beyond actions based on standard clinical practice,” we have little knowledge on the benefits

¹Department of Medicine, University of California, San Diego, La Jolla, CA 92023, USA

²Division of Pulmonary, Critical Care and Sleep Medicine, University of California, San Diego, La Jolla, CA 92023, USA

³Division of Endocrinology, University of California, San Diego, La Jolla, CA 92023, USA

⁴Division of Cardiology, University of California, San Diego, La Jolla, CA 92023, USA

⁵VA San Diego Health System, 9500 Gilman Drive, La Jolla, CA 92023, USA

*Correspondence: amalhotra@health.ucsd.edu (A.M.), jshyy@health.ucsd.edu (J.Y.-J.S.)

<https://doi.org/10.1016/j.isci.2020.101425>



or risks of other drugs known to modulate ACE2, such as anti-diabetic agents. Amid the comorbidity of diabetes and high body mass index (BMI) in COVID-19, this information is urgently needed. As first-line anti-diabetic agents, the biguanide class of drugs (metformin is the only available currently) inhibit gluconeogenesis. Although clinical evidence demonstrating the effect of metformin in RAAS homeostasis in humans is lacking, there are plenty of data from animal and cultured cell experiments supporting that metformin antagonizes the effects of the ACE1–Ang II arm of RAAS (Zhang et al., 2018). Furthermore, large cohort studies have suggested that metformin offers pleiotropic cardiovascular protection. In the United Kingdom Prospective Diabetes Study (UKPDS), metformin therapy in obese patients with type II diabetes was associated with a lower risk for myocardial infarction compared with control (UK Prospective Diabetes Study UKPDS Group, 1998). In an observational study of patients with diabetes and high cardiovascular risk, all-cause mortality was significantly reduced in metformin users compared with non-users, although curiously there were no differences in hard cardiovascular endpoints (Bergmark et al., 2019). Cardiovascular complications, such as non-ischemic cardiomyopathy, have been described as a complication of COVID-19, but the mechanisms involved, including the potential role of ACE2 depletion, remain unclear.

Beyond the potential cardiovascular benefits, several studies have suggested metformin may provide pulmonary protection following SARS-CoV-2 infection. Lung injury, more commonly referred to as ARDS, is one of the most serious complications of COVID-19. Interestingly, metformin has been shown to attenuate lung injury in animal models of lipopolysaccharide (Jian et al., 2013), hyperoxia (Chen et al., 2015), and ventilator-induced lung injury (Tsaknis et al., 2012). Other potential mechanisms include metformin alteration of the microbiome or mitochondrial function (Soberanes et al., 2019; Ouyang et al., 2020). In a retrospective study of human subjects, Jo et al. showed an interesting trend in ARDS mortality comparing metformin users with non-users (42.4% versus 55.3%, $p = ns$), even though metformin was discontinued on admission in the cohort examined (Jo et al., 2017). To our knowledge, metformin has not been studied prospectively in the treatment of ARDS, but these pre-clinical and retrospective data are intriguing.

The molecular basis for the beneficial effects of metformin on the cardiovascular and pulmonary systems likely involves metformin activation of AMP-activated protein kinase (AMPK) (Zhang et al., 2018). Functioning as an energy gauge, AMPK regulates metabolic homeostasis from cellular to whole body levels. AMPK phosphorylates not only key molecules regulating metabolism (e.g., acetyl-CoA carboxylase, sterol regulating element binding proteins) but also those involved in cardiovascular health (e.g., eNOS). Metformin and ARBs (e.g., telmisartan) can activate AMPK, which phosphorylates ACE2 on Ser-680 (Zhang et al., 2018; Shang et al., 2016). This post-translational modification of ACE2 decreases its ubiquitination and therefore extends the half-life of ACE2, which may offer lung protection. Indeed, transgenic mice overexpressing the phosphomimetic ACE2 S680D exhibit less damage in pulmonary vasculature under injurious conditions (Zhang et al., 2018), and ARBs were found to attenuate lung injury after exposure to the S protein of SARS-CoV-1 (Kuba et al., 2005). Owing to similar interactions between ACE2 to SARS-CoV-1 and SARS-CoV-2, it is reasonable to suspect that metformin may offer similar lung protection in COVID-19. A second notion is that post-translational modifications may change the 3-D conformation of the extracellular domain of ACE2, thereby decreasing SARS-CoV-2 viral recognition.

Although diabetes is considered a risk factor for COVID-19, prior clinical studies have suggested diabetic patients may have less risk of lung injury than matched controls (Yu et al., 2013). The reason for this discrepancy is unclear, but at least in theory diabetes medications, such as metformin, could offer protection from ARDS. However, a recent meta-analysis demonstrated conflicting results (Ji et al., 2019) and the possible protective effect of diabetes appears to be present in both type 1 and type 2 DM, suggesting that metformin use was not the underlying mechanism (since metformin is not generally used in type 1 DM). Nonetheless, we believe that the use of metformin to prevent ARDS in the COVID-19 context deserves further study. The use of metformin in critically ill patients is complex, since rare reports of metformin-induced lactic acidosis exist. However, such anecdotes would not preclude a preventative strategy using metformin assuming risk/benefit data were supportive.

We hypothesize that patients with COVID-19 taking metformin will have higher circulating ACE2 levels and lower morbidity and mortality compared with matched controls. We propose to test this hypothesis through a combination of retrospective cohort studies and prospective translational studies evaluating the ACE2 axis in patients with COVID-19. First, the effect of metformin on cardiopulmonary outcomes needs to be evaluated in a cohort of patients with COVID-19. These data could potentially be evaluated

retrospectively in databases that are currently available. Next, we would evaluate circulating ACE2 levels and both arms of the RAAS axis prospectively in patients admitted with COVID-19. These data would allow an assessment of the effect of metformin on circulating ACE2 levels, as well as the ability of circulating ACE2 levels to predict morbidity and mortality due to COVID-19. Noticeably, metformin induction of ACE2 in the cardiovascular system is mainly validated in rodent experiments. The experimental doses used in rats or mice (i.e., 100–200 mg/kg/day) are likely correspondent to the therapeutic doses in human (800–1,600 mg/day). Thus, these mechanisms described are expected to be observed at diabetic doses of metformin therapy. If our hypothesis is supported by these studies, it would provide a strong foundation for rational investigations, including clinical trials, focused on metformin as a safe and immediately available treatment for patients infected with SARS-CoV-2. While this work was submitted for review, we noted an observational study from Wuhan, China, which demonstrated lower hospital mortality in patients with COVID-19 who were being treated for diabetes with metformin, compared with patients not receiving metformin (Luo et al., 2020).

Limitations of the Study

We acknowledge the need for clinical corroboration of our discussion. Despite some potentially compelling rationale, we acknowledge a number of unanswered questions:

1. Does metformin attenuate the risk of poor cardiopulmonary outcomes associated with DM with and without obesity in COVID-19?
2. Does metformin increase circulating ACE2 levels, and do circulating ACE2 levels predict outcomes in patients with COVID-19?
3. Is metformin therapy a safe and effective treatment for patients infected with SARS-CoV-2 who were not previously taking metformin?
4. Does metformin treatment have clinical benefit in patients with COVID-19 who are not diabetic or obese?

In summary, ACE2 is an anti-inflammatory peptide in the protective arm of the RAAS system that offers beneficial cardiopulmonary effects. Although binding of ACE2 receptors and subsequent downregulation appears to be a key feature in the pathophysiology of SARS-CoV-1 and SARS-CoV-2 infections, therapies that enhance ACE2 have great potential to benefit patients suffering from COVID-19. Metformin is a commonly used antidiabetic agent that is known to enhance ACE2 expression via AMPK activation. Metformin has an excellent safety profile and wide availability throughout the world, which allows for immediate, wide distribution if proven to be effective as a monotherapy, or in combination with antiviral agents, such as remdesivir.

REFERENCES

- Chen, X., Walther, F.J., Sengers, R.M., Laghmani, E.H., Salam, A., Folkerts, G., Pera, T., and Wagenaar, G.T. (2015). Metformin attenuates hyperoxia-induced lung injury in neonatal rats by reducing the inflammatory response. *Am. J. Physiol. Lung Cell Mol. Physiol.* 309, L262–L270.
- Bergmark, B.A., Bhatt, D.L., McGuire, D.K., Cahn, A., Mosenzon, O., Steg, P.G., Im, K., Kanevsky, E., Gurmu, Y., Raz, I., et al. (2019). Metformin use and clinical outcomes among patients with diabetes mellitus with or without Heart failure or kidney dysfunction: observations from the SAVOR-TIMI 53 trial. *Circulation* 140, 1004–1014.
- Chen, A.T.C., Coura-Filho, G.B., and Rehder, M.H.H. (2020). Clinical characteristics of covid-19 in China. *N. Engl. J. Med.* 382, 1859–1862.
- Ferrario, C.M., Jessup, J., Chappell, M.C., Averill, D.B., Brosnihan, K.B., Tallant, E.A., Diz, D.J., and Gallagher, P.E. (2005). Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation* 111, 2605–2610.
- Ji, M., Chen, M., Hong, X., Chen, T., and Zhang, N. (2019). The effect of diabetes on the risk and mortality of acute lung injury/acute respiratory distress syndrome: a meta-analysis. *Medicine (Baltimore)* 98, e15095.
- Jian, M.Y., Alexeyev, M.F., Wolkowicz, P.E., Zmijewski, J.W., and Creighton, J.R. (2013). Metformin-stimulated AMPK- α 1 promotes microvascular repair in acute lung injury. *Am. J. Physiol. Lung Cell Mol. Physiol.* 305, L844–L855.
- Jo, Y.S., Choi, S.M., Lee, J., Park, Y.S., Lee, C.H., Yim, J.J., Yoo, C.G., Kim, Y.W., Han, S.K., and Lee, S.M. (2017). Effect of preadmission metformin use on clinical outcome of acute respiratory distress syndrome among critically ill patients with diabetes. *Tuberc. Respir. Dis. (Seoul)* 80, 296–303.
- Kuba, K., Imai, Y., Rao, S., Gao, H., Guo, F., Guan, B., Huan, Y., Yang, P., Zhang, Y., Deng, W., and Bao, L. (2005). A crucial role of angiotensin-converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat. Med.* 11, 875–879.
- Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., Zhang, Q., Shi, X., Wang, Q., Zhang, L., and Wang, X. (2020). Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature* 581, 215–220.
- Luo, P., Lin, Q., Liu, Y., Liu, X.L., Zheng, J.L., Xue, H.Y., Liu, W.H., Liu, D., and Li, J. (2020). Metformin treatment was associated with decreased mortality in COVID-19 patients with diabetes in a retrospective analysis. *Am. J. Trop. Med. Hyg.* 103, 69–72.
- Monteil, V., Kwon, H., Prado, P., Hagelkrüys, A., Wimmer, R.A., Stahl, M., Leopoldi, A., Garreta, E., Del Pozo, C.H., Prosper, F., and Romero, J.P. (2020). Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE₂. *Cell* 181, 905–913.e7.
- Ouyang, J., Isnard, S., Lin, J., Fombuena, B., Marette, A., Routy, B., Chen, Y., and Routy, J.P.

(2020). Metformin effect on gut microbiota: insights for HIV-related inflammation. *AIDS Res. Ther.* 17, <https://doi.org/10.1186/s12981-020-00267-2>.

Ramchand, J., Patel, S.K., Srivastava, P.M., Farouque, O., and Burrell, L.M. (2018). Elevated plasma angiotensin converting enzyme 2 activity is an independent predictor of major adverse cardiac events in patients with obstructive coronary artery disease. *PLoS One* 13, e0198144.

Reddy, R., Asante, I., Liu, S., Parikh, P., Liebler, J., Borok, Z., Rodgers, K., Baydur, A., and Louie, S.G. (2019). Circulating angiotensin peptides levels in Acute Respiratory Distress Syndrome correlate with clinical outcomes: a pilot study. *PLoS One* 14, e0213096.

Santos, R.A.S., Sampaio, W.O., Alzamora, A.C., Motta-Santos, D., Alenina, N., Bader, M., and Campagnole-Santos, M.J. (2018). The ACE2/angiotensin-(1-7)/MAS Axis of the renin-angiotensin system: focus on angiotensin-(1-7). *Physiol. Rev.* 98, 505–553.

Shang, F., Zhang, J., Li, Z., Zhang, J., Yin, Y., Wang, Y., Marin, T.L., Gongol, B., Xiao, H., and Zhang, Y.Y. (2016). Chen Z cardiovascular protective effect of metformin and telmisartan: reduction of PARP1 activity via the AMPK-PARP1 cascade. *PLoS One* 11, e0151845.

Soberanes, S., Misharin, A.V., Jairaman, A., Morales-Nebreda, L., McQuattie-Pimentel, A.C., Takugo, C., Hamanaka, R.B., Meliton, A.Y., Reyfman, P.A., Walter, J.M., et al. (2019). Metformin targets mitochondrial electron transport to reduce air-pollution-induced thrombosis. *Cell Metab.* 29, 335–347.e5.

Tsaknis, G., Siempos, I., Kopterides, P., Maniatis, N.A., Magkou, C., Kardara, M., Panoutsou, S., Kotanidou, A., Roussos, C., and Armaganidis, A. (2012). Metformin attenuates ventilator-induced lung injury. *Crit. Care* 16, R134.

UK Prospective Diabetes Study (UKPDS) Group (1998). Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *UK*

Prospective Diabetes Study (UKPDS) Group. *Lancet* 352, 837–853.

Vaduganathan, M., Vardeny, O., Michel, T., McMurray, J.J.V., Pfeffer, M.A., and Solomon, S.D. (2020). Renin-angiotensin-aldosterone system inhibitors in patients with covid-19. *N. Engl. J. Med.* 382, 1653–1659.

Wrapp, D., Wang, N., Corbett, K.S., Goldsmith, J.A., Hsieh, C.L., Abiona, O., Graham, B.S., and McLellan, J.S. (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 367, 1260–1263.

Yu, S., Christiani, D.C., Thompson, B.T., Bajwa, E.K., and Gong, M.N. (2013). Role of diabetes in the development of acute respiratory distress syndrome. *Crit. Care Med.* 41, 2720–2732.

Zhang, J., Dong, J., Martin, M., He, M., Gongol, B., Marin, T.L., Chen, L., Shi, X., Yin, Y., Shang, F., and Wu, Y. (2018). AMP-activated protein kinase phosphorylation of angiotensin-converting enzyme 2 in endothelium mitigates pulmonary hypertension. *Am. J. Respir. Crit. Care Med.* 198, 509–520.