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Review Article

Correcting the imbalanced protective RAS in COVID-19 with angiotensin AT_2-receptor agonists

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that is responsible for the global corona virus disease 2019 (COVID-19) pandemic enters host cells via a mechanism that includes binding to angiotensin converting enzyme (ACE) 2 (ACE2). Membrane-bound ACE2 is depleted as a result of this entry mechanism. The consequence is that the protective renin–angiotensin system (RAS), of which ACE2 is an essential component, is compromised through lack of production of the protective peptides angiotensin-(1-7) and angiotensin-(1-9), and therefore decreased stimulation of Mas (receptor Mas) and angiotensin AT_2-receptors (AT_2Rs), while angiotensin AT_1-receptors (AT_1Rs) are overstimulated due to less degradation of angiotensin II (Ang II) by ACE2. The protective RAS has numerous beneficial actions, including anti-inflammatory, anti-coagulative, anti-fibrotic effects along with endothelial and neural protection; opposite to the deleterious effects caused by heightened stimulation of angiotensin AT_1R. Given that patients with severe COVID-19 exhibit an excessive immune response, endothelial dysfunction, increased clotting, thromboses and stroke, enhancing the activity of the protective RAS is likely beneficial. In this article, we discuss the evidence for a dysfunctional protective RAS in COVID and develop a rationale that the protective RAS imbalance in COVID-19 may be corrected by using AT_2R agonists. We further review preclinical studies with AT_2R agonists which suggest that AT_2R stimulation may be therapeutically effective to treat COVID-19-induced disorders of various organ systems such as lung, vasculature, or the brain. Finally, we provide information on the design of a clinical trial in which patients with COVID-19 were treated with the AT_2R agonist Compound 21 (C21). This trial has been completed, but results have not yet been reported.

Introduction

The discovery in 2003 of angiotensin converting enzyme (ACE) 2 (ACE2) as the binding site and cellular entry point for the severe acute respiratory syndrome coronavirus (SARS-CoV) unexpectedly pointed to a link between coronavirus infections and the renin–angiotensin system (RAS) [1]. The current global Corona Virus Disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which also uses ACE2 as a cellular entry point [2,3], has revived interest and research in this area, resulting in thousands of publications during the past 11 months.

ACE2 is highly expressed in lung alveolar type II and nasal epithelia [4], and therefore these cells serve as main entry points for SARS-CoV-2 into the body. Many of the individuals who contract SARS-CoV-2 are either asymptomatic, or experience mild, common cold-like symptoms and recover soon. However, ∼20% of patients develop more serious COVID-19 disease, which is often driven by an excessive immune response termed as a 'cytokine storm' [5]. In most cases, these patients develop pneumonia, which can lead to respiratory failure and acute respiratory distress syndrome (ARDS) [6]. Many patients also experience extra-pulmonary manifestations affecting heart, kidneys, brain and other organs with multi-organ failure

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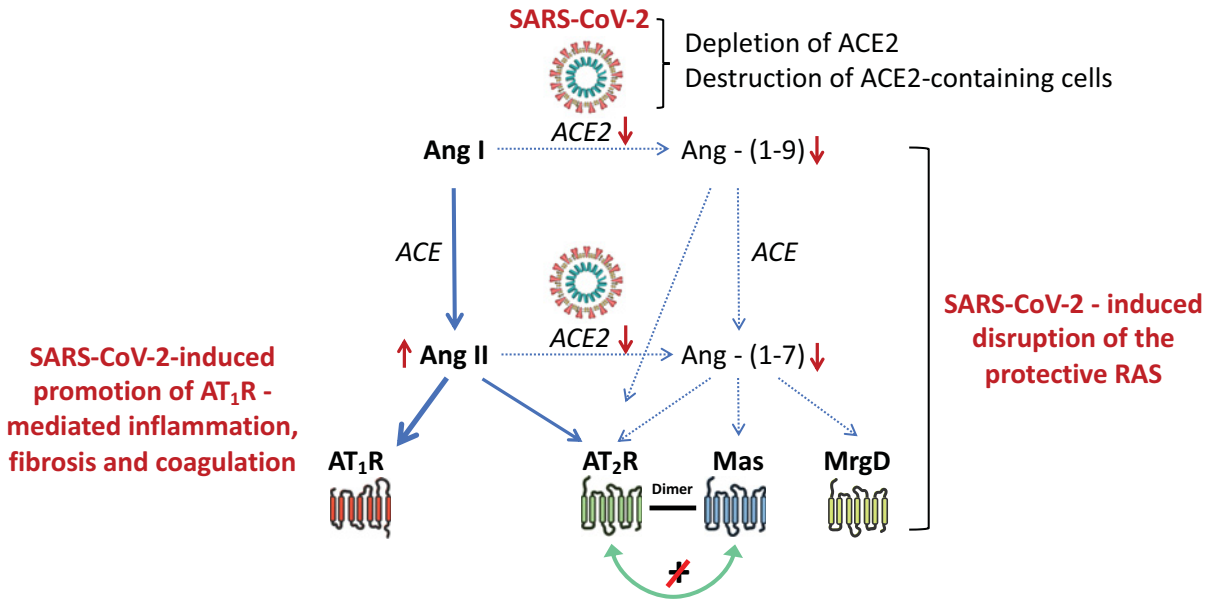

Figure 1. Disruption of the protective RAS through SARS-CoV-2

This diagram depicts the ways in which SARS-CoV-2 infection can lead to disruption of the protective arm of the RAS. This disruption involves: depletion of ACE2, decreased Ang-(1-7) and Ang-(1-9) (red downward arrows) with consequently less activation of Mas, AT_2R and MrgD receptors; and a lack of Mas-induced increases in AT_2R expression, and *vice versa* (green arrow). At the same time, an abundance of Ang II will enhance AT_1R-mediated inflammation, coagulation and fibrosis (red upward arrow). Abbreviations: Ang-(1-7), angiotensin-(1-7); Ang-(1-9), angiotensin-(1-9); AT_1R, angiotensin AT_1-receptor; AT_2R, angiotensin AT_2-receptor; Mas, receptor Mas; MrgD, Mas-related G-protein coupled receptor member D. SARS-CoV-2 image credit: *Desiree Ho* for the Innovative Genomics Institute (https://innovativegenomics.org/free-covid-19-illustrations/).

in the worst case [7]. These complications are not only a result of direct SARS-CoV-2 infection, but also of a systemic endotheliitis causing coagulopathy and thromboembolic complications [8–10].

Given the current worldwide death rate of patients with COVID-19 of ∼3.0% and the unavailability of a specific treatment as yet, there is a clear need for novel therapies. Such novel therapies for COVID-19 may reside in the RAS. When considering the interactions between the SARS coronaviruses (SARS-CoVs) and the RAS with regard to the development of potential new drugs for COVID-19, there are several aspects that are important.

One aspect is the mechanisms by which these viruses bind to ACE2 and their subsequent entry into human cells. Such knowledge is particularly important because it may guide the way to the development of novel drugs that are able to prevent SARS-CoVs' cellular entry through inhibition of binding to ACE2.

Furthermore, the SARS-CoVs/ACE2 interaction leads to changes within the expression and ratio of other RAS components, which are most likely of relevance for the severity of organ injury (Figure 1) [11]. This includes on the one hand a rise in angiotensin II (Ang II) levels and its stimulatory and potentially harmful effects on inflammation, coagulation and fibrosis via the AT_1R, and on the other hand a shortage in protective mediators and mechanisms of the so-called protective arm of the RAS [11,12]. This imbalance of the RAS in SARS-CoVs infection will be discussed thoroughly later in this review. The impairment of the protective arm of the RAS in COVID-19 patients is the rationale for several drug development projects and clinical trials. One such trial has tested safety and efficacy of an agonist (Compound 21, C21) for the angiotensin AT_2-receptor (AT_2R), which is a receptor of the protective arm of the RAS, in hospitalised patients with COVID-19 infection who do not require intensive care (**A**ngiotensin II **T**ype **T**wo **R**eceptor **A**gonist in **C**OVID-19 **T**rial (ATTRACT) study) [13]. The trial has been completed, but results have not yet been reported.

This article will discuss how SARS-CoV-2 infection, ACE2 and AT_2R biology and function are related. It will also discuss interactions between the main receptors of the protective RAS, AT_2R and Mas, and how this cross-talk may play a role in COVID-19 pathology and treatment. We will further lay out the reasons why AT_2R stimulation may be effective in patients with COVID-19 infection, and provide information on the design of the clinical trial with C21 in COVID-19 patients.

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The protective RAS

Aside from the well-known classical RAS with Ang II acting on the AT_1R as its main functional entity, there also exists a so-called 'protective' or 'alternate' RAS that in general opposes the actions of the classical RAS [14]. The main mediators of the protective RAS are angiotensin-(1-7) [Ang-(1-7)], angiotensin-(1-9) [Ang-(1-9)], alamandine, Ang II and angiotensin III (Ang III), and the main receptors are AT_2R, Mas, and Mas-related G-protein coupled receptor member D (MrgD) (Figure 1) [14].

ACE2 is one of the main enzymes of the RAS, generating Ang-(1-7) directly from Ang II and Ang-(1-9) from Ang I, which is then further processed by ACE to Ang-(1-7). Importantly, ACE2 does not only cause an increase in Ang-(1-7) and Ang-(1-9) levels, but it also leads to a decrease in the substrates, Ang I and Ang II [15]. Ang-(1-7) exerts its protective actions by binding to the receptor Mas [16] and also as a β-arrestin biased agonist at the AT_1R [17,18]. Alamandine is generated from Angiotensin A, catalysed by ACE2, and is an agonist for the MrgD receptor. Ang III is an endogenous agonist for the AT_2R in peripheral tissues [19], but likely an AT_1R agonist in the brain [20]. Ang II is a potent agonist for both, the AT_1R and the AT_2R, but with slightly higher affinity for the AT_2R. Nevertheless, since in the vast majority of tissues and cells AT_1R expression is much higher than that of AT_2R, a net Ang II response is usually a result of AT_1R stimulation.

Both Mas and MrgD mediate protective actions that have been reviewed extensively elsewhere, with the actions of Ang-(1-7) via Mas particularly well-defined [21]. These actions of Ang-(1-7) via Mas include potent anti-inflammatory, anti-oxidative, anti-proliferative, and anti-fibrotic actions, as well as inducing endothelial- and neuroprotection [22]. Thus, a decline or loss of these protective Ang-(1-7) actions in COVID-19 may help exacerbate the disease state. On the other hand, activation of AT_2R exerts very similar powerful protective actions as Ang-(1-7) at both the systemic and brain levels, and so AT_2R agonists may substitute for the loss of natural Ang-(1-7) protective mechanisms in COVID-19 disease. These AT_2R actions are discussed in more detail in later sections of this review.

Interaction of the AT_2R with other components of the protective RAS

The interactions of the AT_2R with other components of the protective RAS are multifaceted. As will be discussed in detail in later paragraphs of this review, these interactions are important to understand the rationale of AT_2R-agonists for the treatment of COVID-19.

In general, according to current data it seems that the different arms of the protective RAS, i.e. those connected to the AT_2R and those connected to Mas/MrgD, reinforce each other by various ways of cross-talk. The main examples of this type of cross-talk are discussed in the following paragraphs:

Effect of AT_2R stimulation on the expression of other protective RAS components

There are multiple publications reporting that AT_2R stimulation leads to an increase in the expression of other protective RAS components thus constituting a type of positive feedback mechanism within the protective RAS: for example, stimulation of AT_2R leads to an increase in ACE2 expression and subsequently an increase in the protective RAS hormones Ang-(1-7) and Ang-(1-9). To name some examples, an AT_2R-mediated increase in ACE2 expression was demonstrated in the renal cortex of male, obese rats on a high-salt diet [23] and of male obese Zucker rats [24], in the plasma of type I diabetic rats with ischaemic renal injury [25], and, importantly, in fibrotic lungs in a monocrotaline model in mice [26].

An AT_2R-mediated increase in Mas expression was, for example, reported in the renal cortex of obese Zucker rats and in lungs of rats after monocrotaline challenge [24,26].

This positive reinforcement within the protective RAS also works in the other direction with Mas-stimulation resulting in increased AT_2R expression as, for example, shown in injured femoral arteries of rats treated with Ang-(1-7) [27].

Aligned regulation of protective RAS components

Apart from direct regulation of ACE2 by AT_2R/Mas stimulation, there is also evidence in the literature that AT_2R and ACE2 expression is often regulated by various RAS-related or -unrelated compounds in an aligned way. For example, tissue-protective compounds such as AT_1 receptor blockers (ARBs), curcumin [28], glucagon-like peptide-1 receptor agonists or dipeptidyl peptidase-4 inhibitors [29] caused a coincident increase in AT_2R and ACE2 expression/activity. In contrast, harmful compounds such as Ang II, applied at a hypertensive dose in male Sprague–Dawley rats [30],

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led to a decrease in both AT_2R and ACE2 expression/activity. It is tempting to speculate that AT_2R and ACE2 share a common mechanism of gene regulation, which reacts to certain stimuli in the same way; however, this has not been investigated as yet.

AT_2R/Mas dimerisation

Another type of AT_2R-/Mas-axis interaction is the heterodimerisation of AT_2R and Mas. AT_2R/Mas-heterodimerisation has first been shown in rat astrocytes *in vitro* [31] and later in renal proximal tubules and cortical homogenates from obese Zucker rats *ex vivo* and in human proximal tubule epithelial cells *in vitro* [32]. Other organs and cell types have not been tested yet for AT_2R/Mas-heterodimerisation, and consequently knowledge about the occurrence and significance of AT_2R/Mas-heterodimerisation is still fragmentary. What is known is that at least in some tissues, in which AT_2R and Mas form heterodimers, functionality of one receptor depends on the presence of the other receptor [31,32].

With regard to cross-talk between the AT_2R-related and the Mas-related arms of the protective RAS, AT_2R/Mas-heterodimerisation could cause that AT_2R-agonists also stimulate Mas and Mas-agonists also stimulate the AT_2R. Moreover, heterodimerisation seems to cause cross-inhibition, which means that AT_2R effects can be blocked by a Mas antagonists and Mas effects by an AT_2R antagonist [31,32].

Effects of Ang-(1-7) and Ang-(1-9) at the AT_2R

Ang-(1-7) and Ang-(1-9) are both products resulting from ACE2 enzymatic activity with Ang-(1-7) derived from cleavage of Ang II and Ang-(1-9) derived from cleavage of Ang I [22]. In the context of physiological and pathophysiological actions of ACE2, including its role in COVID-19, focus is usually laid on the ACE2/Ang-(1-7)/Mas axis [11]. However, ACE2 enzymatic products are not only agonists for the receptor Mas, but also for the AT_2R. Specifically, Ang-(1-7) binds to the AT_2R with low affinity and has agonistic properties at this receptor [33,34], and Ang-(1-9) has been described as an endogenous AT_2R agonist [35]. However, it is not entirely clear, whether actions of Ang-(1-9) are really elicited by this hormone or whether the active component is actually its degradation product, Ang-(1-7).

The fact that Ang-(1-7) and potentially Ang-(1-9) have agonistic properties at the AT_2R obviously means that in case of changes in ACE2 expression and enzymatic activity such as in COVID-19, not only signalling and effects mediated by the receptor Mas are affected, but also signalling and effects mediated by the AT_2R.

In summary, there are several mechanisms, by which an alteration of one RAS component can have an impact on the expression or functionality of other RAS components. The following section will discuss the potential nature of such interactions, specifically how reduced ACE2 expression in COVID-19 may lead to changes within the protective RAS and how this may impact the course of the disease.

Imbalance of the protective RAS in COVID-19

As discovered by Li et al. in 2003 for SARS-CoV [1] and by Zhou et al. and Wan et al. in 2020 for SARS-CoV-2 [2,3], SARS viruses bind to extracellular portions of ACE2 with their spike (S) glycoprotein. The process of SARS-CoVs/ACE2 binding is facilitated through S-protein priming by transmembrane protease serine 2 (TMPRSS2) [36,37]. This protease is a potential drug target for the treatment of COVID-19, since inhibitors of TMPRSS2, camostat mesylate and nafamostat mesylate, successfully prevented SARS-CoV-2 cell entry into cells [37,38].

After binding of SARS-CoV viruses to ACE2, the virus/enzyme complex is internalised, which leads to loss of enzymatically active ACE2 at the cell surface [37,39,40].

The loss of ACE2 enzymes and ACE2 enzymatic activity is the key event that triggers the resulting imbalance within the RAS. There are two main initial consequences of a loss of ACE2 activity: (1) decreased synthesis of the protective mediators Ang-(1-7), Ang-(1-9) and alamandine, and (2) increased levels of Ang II due to less degradation by ACE2 [11]. Or in other words, there is a weakening of the protective arm of the RAS and a strengthening of the potentially harmful, classical arm of the RAS.

Experimental and clinical evidence for an imbalanced RAS in SARS-CoV infection and consequences for the course of disease

As stated above, current knowledge about ACE2 suggests that in case of a SARS-CoV infection and a resulting decrease in ACE2 activity, Ang II levels will rise, while levels of Ang-(1-7) [and also Ang-(1-9)] will decline. In the following, we review studies which tested the above assumption about a RAS imbalance in COVID and whether such an imbalance does have any impact on the course of the disease.

The strongest evidence still comes from studies performed in relation to the first SARS-CoV/COVID outbreak in 2003. A landmark study in this regard is a publication from Josef Penninger's group from 2005. In this study, the authors first demonstrated the crucial role of ACE2 for the process of SARS-CoV infection [39]. They found that ACE2-knockout mice presented with a much lower pulmonary viral load than wild-type mice on day 2 after intranasal inoculation with 100 μl SARS-CoV virus, thus supporting the importance of ACE2 as an entry gate for the SARS-CoV virus. In further experiments within this study, the authors used a recombinant SARS-CoV surface-Spike protein instead of the intact virus after showing that the Spike-Fc protein binds to ACE2 and reduces ACE2 surface expression *in vitro*. After Spike-Fc protein 'infection' of C57Bl/6 mice, which were additionally challenged by acid aspiration to induce acute lung injury (ALI), the authors indeed reported an imbalance of the RAS by showing that pulmonary Ang II levels, which were already elevated after acid aspiration, increased further by Spike-Fc protein infection [39]. Infection with the Spike-Fc protein and elevated Ang II levels coincided with a more severe course of ALI in these mice. Moreover, the fact that pharmacological AT_1R blockade with losartan (15 mg/kg) lessened the severity of symptoms of ALI in these mice clearly supported the hypothesis that elevated Ang II levels caused by virus-induced lowering of ACE2 expression and acting via the Ang II/AT_1R axis play a crucial role for the severity of disease [39].

In another study from the same group, the authors showed in three different, non-viral models of ALI and ARDS (models of ALI induced by acid aspiration, sepsis or endotoxins) that the pathological changes within the lung, such as reduced lung elastance and pulmonary edema formation, were more severe and mortality increased in ACE2-deficient mice (a condition resembling SARS-CoV-induced ACE2 inactivation) compared with wild-type mice [41]. The responsibility of ACE2 deficiency for these differences was further substantiated by rescue experiments, in which application of human recombinant ACE2 significantly attenuated the severity of lung injury and dysfunction in ACE2-knockout mice, an effect that was not seen after application of mutated, non-functional recombinant ACE2 molecules.

This study also provided important evidence that ACE2 deficiency indeed causes an increase in Ang II levels by demonstrating elevated Ang II content in lungs of ACE2-knockout mice when compared with wild-type mice in the acid aspiration model [41]. Moreover, similar to the situation in the study with Spike-Fc protein infection, pharmacological AT_1R blockade lessened the severity of symptoms of ALI in ACE2-knockout mice, thus supporting the detrimental role of elevated Ang II levels caused by ACE2 deficiency. Interestingly in the context of this article, the authors also concluded that the AT_2R protects against lung injury; however the only data provided in support were the lack of reversal of severe lung injury in ACE2-KO mice by pharmacological AT_2R blockade [41].

Taken together, these studies by the Penninger group provided strong support for the hypothesis that elevated Ang II levels as a result of virus-induced down-regulation of ACE2 are an important driver of SARS-CoV induced lung injury. A piece of information that was missing in these studies and would have rounded out the story was a proof for the impairment of the protective arm of the RAS, specifically lower levels of Ang-(1-7) and Ang-(1-9) in lungs of animals infected with the SARS-CoV virus, with the Spike-Fc protein or in ACE2-deficient mice.

It is still not really clear how much the imbalance of the RAS as predicted from theoretical considerations and as observed in animal models actually occurs in humans infected with a SARS-CoV virus, and what the impact of such an imbalance on the course of the disease in humans might be.

A few first studies have been published in which plasma concentrations of RAS components in COVID-19 patients were determined during the current outbreak. These studies elicited conflicting results.

One study by Wu et al. measured plasma Ang II levels in 82 patients with COVID-19 and 12 patients who were severely ill from other diseases, and found a significant increase in Ang II levels in COVID-19 patients, which correlated with the severity of the disease [42]. Measurement of Ang II was likely by enzyme-linked immunosorbent assay (ELISA), but no details about methodology were reported. Liu et al. also reported significantly increased Ang II plasma levels in 12 patients with COVID-19, which correlated positively with viral load and lung injury [43]. In contrast, Henry et al. did not note any difference in plasma Ang II levels between 30 patients with COVID-19 and 12 healthy controls [44]. There were also no differences between hospitalised and discharged patients or between patients requiring intensive care unit support or not. The latter two studies used ELISA for the measurement of plasma Ang II.

A very recent study by Kintscher et al. determined plasma angiotensin peptides by LC-MS/MS [45]. Plasma samples were derived from patients with or without COVID-19 admitted to the emergency room. This study also did not find any significant differences in angiotensin peptide levels or ACE2 activity between patients with or without COVID-19. However, as the authors state themselves, the study had several limitations including a low number of patients (n=8–12 per group) and therefore insufficient statistical power.

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It is a limitation of all four studies that they only measured Ang II levels in plasma, which do not necessarily reflect the situation in infected tissues like the upper respiratory tract and the lungs.

Mechanisms of weakening the protective actions of the AT_2R by SARS-CoV-2 infection

While it is discussed many times in the recent literature on RAS/COVID-19 interactions that a weakening of ACE2 enzymatic activity leads to a decrease in synthesis of Ang-(1-7) resulting in reduced stimulation of the receptor Mas and a weakening of the protective effects through β-arrestin-biased agonism at the AT_1R, there is much less awareness that decreased ACE2 enzymatic activity also leads to reduced stimulation of the AT_2R. This is for several reasons, which are illustrated in Figure 1 and are as follows:

- less synthesis of the putative AT_2R-agonist Ang-(1-9),
- less synthesis of Ang-(1-7), which is not only a Mas, but also a low-affinity AT_2R-agonist,
- less AT_2R-stimulation by Ang-(1-7) through cross-activation of Mas-AT_2R-heterodimers, and
- less induction of AT_2R-expression by Ang-(1-7) acting on the receptor Mas.

It is also important to note that decreased ACE2 enzymatic activity in COVID-19 would lead to increased levels of the ACE2 substrate Ang II, that would conceivably stimulate the protective RAS via activation of AT_2R. However, the benefits of such AT_2R activation by Ang II would largely be overridden or offset by its simultaneous activation of the more predominant AT_1R pathways. Thus, application of a selective AT_2R agonist (or Mas agonist) would be required to stimulate the protective RAS in COVID-19.

Rationale for AT_2R agonists in COVID-19

One of many current attempts to find a treatment for patients with COVID-19 is to strengthen the protective arm of the RAS, which is impaired in a multifaceted way by infection of cells with the SARS-CoV-2 virus as reviewed above. Three such approaches, which are human recombinant ACE2 [46], formulations of Ang-1-7 (e.g. TXA127) [47] or an AT_2R agonist [13] are currently being tested in clinical trials in patients with COVID-19.

The following will discuss the rationale for AT_2R agonists in COVID-19 including considerations on how AT_2R-agonists may correct for the above-discussed imbalance within the protective RAS and also on how direct effects of AT_2R stimulation on certain aspects of COVID-19 pathology may have a therapeutic effect in these patients.

Compensation for impairment of the protective RAS

As discussed in the preceding sections, infection of a cell or organism with a SARS-CoV virus leads to a reduction in ACE2 enzymatic activity, resulting in a lessening of the synthesis of the protective RAS mediators Ang-(1-7) and Ang-(1-9) and, subsequently, decreased stimulation of the receptor Mas and the AT_2R [11].

While it is likely that in SARS-CoV-2 infections substituting ACE2 with recombinant human ACE2 or compensating for reduced Ang-(1-7) levels by application of recombinant Ang-(1-7) (or derivatives) may be promising strategies to correct the virus-induced impairment of the protective RAS, it is less obvious why AT_2R-agonists may also be used for this purpose.

As discussed in the section on the imbalance of the protective RAS in SARS-CoV infection, AT_2R stimulation is impaired by various mechanisms. Collectively, these mechanisms weaken AT_2R stimulation and signalling and this can be counteracted by application of AT_2R-agonists. Moreover, AT_2R-agonists may also lead to stimulation of Mas signalling through cross-activation of AT_2R-Mas-heterodimers [31,48].

Anti-inflammatory effects of AT_2R activation

As would be expected, COVID-19 infection elicits activation of the innate and adaptive immune systems, and in a majority of cases their combined actions result in disease resolution [49–53]. In patients with more severe COVID-19 disease, those displaying ARDS and pneumonia, clinical findings indicate that multiple aspects of the innate and adaptive immune systems are compromised or dysregulated [50,51,53]. Of importance for the current article is the observation that this group of COVID-19 patients exhibits massively increased levels of circulating pro-inflammatory cytokines, including interleukin (IL)-1β (IL-1β), IL-6, tumour necrosis factor (TNFα), IL-17 and interferon-γ, (IFN-γ) [53,54]. The resulting chronic pro-inflammatory milieu, termed as a 'cytokine storm', is likely responsible for the uncontrolled inflammation within the lungs, heart, vascular endothelium and kidney, and recruitment of immune cells

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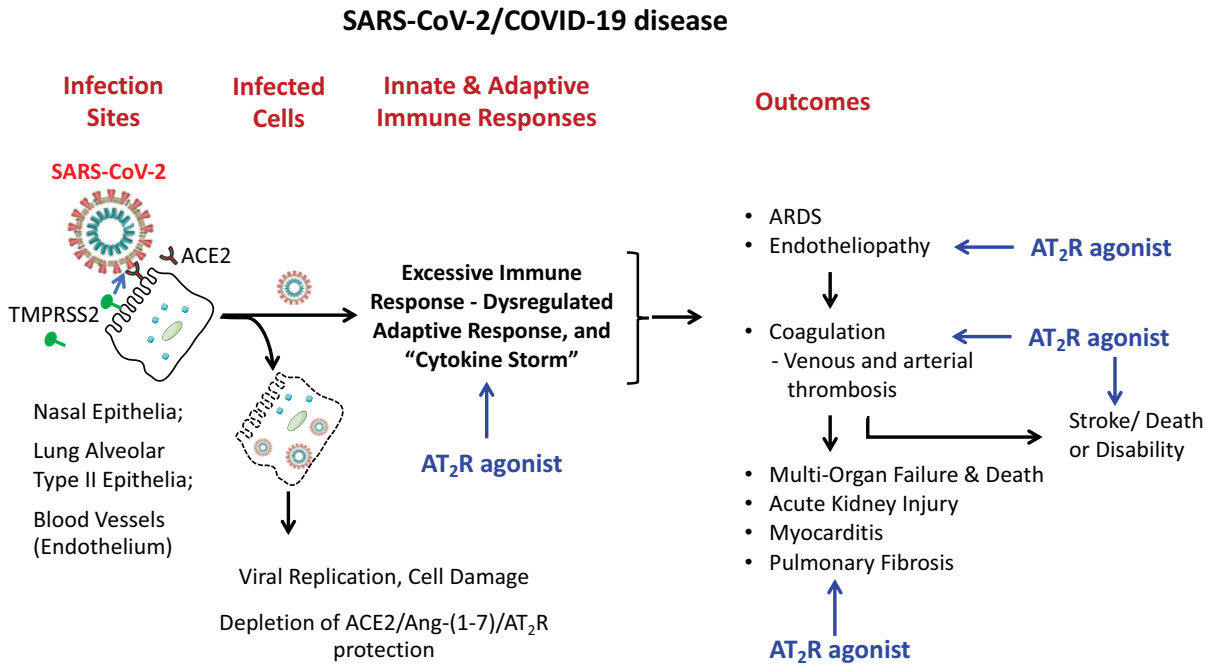

Figure 2. Potential attenuation of severe COVID-19 by AT_2R agonists

This diagram illustrates where AT_2R agonists may be of potential benefit in COVID-19 following SARS-CoV-2 infection; at the anti-inflammatory, endotheliopathy, coagulopathy, stroke and anti-fibrotic levels, as indicated by blue arrows. Abbreviation: AT_2R, angiotensin AT_2-receptor. SARS-CoV-2 image credit: *Desiree Ho* for the Innovative Genomics Institute (https://innovativegenomics.org/free-covid-19-illustrations/). Epithelial cell pictures are reproduced from Servier Medical Art Commons Attribution 3.0 Unported License. (http://smart.servier.com). Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License.

(macrophages, neutrophils, T cells) to these sites [7,55]. The subsequent release of pro-inflammatory cytokines from these cells and generation of reactive oxygen species results in endothelial cell and tissue damage [8,51,56].

As indicated in Figure 2, the excessive immune response in patients with severe COVID-19 may lead to various pathological outcomes: coagulopathy with associated venous and arterial thrombosis, subsequent stroke and possible death or disability [57,58]; multi-organ failure, partly due to coagulopathy, and possible death [59–62]; deleterious effects on vital organs, including acute kidney injury [63,64], myocarditis [65] and pulmonary fibrosis [66–69].

Considering the excessive pro-inflammatory response and the decreased anti-inflammatory capacity of the ACE2/Ang-(1-7) system following COVID-19 infection, anti-inflammatory agents would provide a logical therapeutic strategy [52,70]. Indeed, a number of recent clinical studies have indicated beneficial effects of corticosteroids administered systemically to patients with ARDS in severe COVID-19 disease [71].

It is well-known that AT_2R agonists exert powerful anti-inflammatory actions [72–75]; thus, they may be prime candidates to compensate for the loss of ACE2/Ang-(1-7) protective mechanisms and help offset the large pro-inflammatory response in COVID-19 patients with severe disease (Figure 2). Based on the literature available, the anti-inflammatory effects associated with AT_2R may result from direct effects on immune system cells, as well as effects on non-immune cells.

Direct anti-inflammatory AT_2R actions via cells of the innate and adaptive immune systems

Innate immune system

In general, initial COVID-19 infection will result in activation of the innate immune system, including monocytes, macrophages, dendritic cells and the complement system [50,51,53]. Human monocytes, macrophages and dendritic cells contain AT_2R [75–77] and a number of studies indicate that activation of this angiotensin receptor subtype interferes with certain aspects of the innate immune system response. For example, *in vitro* studies have demonstrated that the AT_2R agonist CGP42112 inhibits the IL-1β induced activation of monocytes [78]. Furthermore, a number

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of studies indicate that AT_2R activation interferes with Toll-Like Receptor (TLR) – induced pro-inflammatory mechanisms. TLR-induced activation of macrophages is well established [79], and in the case of COVID-19 infection this involves endosomal TLR3 and TLR7/8, as well as the indirect activation of cell-surface TLR4, via virus-induced oxidised phospholipids [80]. Interestingly, the AT_2R agonist C21 exhibits anti-inflammatory actions in TLR4-mediated inflammation in THP-1 macrophages, mediated via IL-10 production [76]. Another study, using human monocyte cell lines, has demonstrated that inflammatory responses mediated by TLR4 were attenuated by C21 acting to reduce pro-inflammatory cytokines [81]. Of direct importance to the immune pro-inflammatory response occurring after COVID-19 infection, C21 has been shown to exert significant anti-inflammatory effects in the lung, via influences on TLR4 and macrophage infiltration [82]. Specifically, systemic administration of C21 into rats that had undergone bleomycin-induced pulmonary inflammation and fibrosis prevented the up-regulation of TLR4 and lessened the migration of $CD68^+$ macrophages in the lung [82]. In another study, investigators used neonatal rats with hyperoxia-induced lung injury to evaluate the anti-inflammatory effects of an AT_2R agonist. Daily treatment of these rats with dKcAng-(1-7), a specific AT_2R ligand, attenuated the pulmonary influx of macrophages [83]. The anti-inflammatory effects of AT_2R activation are not confined to monocyte/macrophage-mediated mechanisms, as indirect evidence suggests that dendritic cells are another locus of action. Specifically, human dendritic cells that underwent differentiation in the presence of the AT_2R antagonist PD123319, displayed a more pro-inflammatory phenotype [84]. A more recent study indicates that the potent anti-inflammatory effect of ACE inhibitors on dendritic cells post-myocardial infarction is at least partly due to activation of AT_2R [85]. As yet, there is no evidence for modulation of the complement system by AT_2R agonists.

Adaptive immune system

COVID-19 infection also results in activation of the adaptive immune system, albeit over a more delayed time frame [50,51,53]. T helper ($CD4^+$) and cytotoxic ($CD8^+$) T cells are central to the adaptive immune response [86]. Interestingly, a feature of COVID-19 disease is that patients display lymphopaenia, with a reduction in the absolute levels of all types of T cells, of natural killer (NK) cells and also B cells [51,53]. While this lymphopaenia would compromise the adaptive immune response, studies have also demonstrated that the *activity* of $CD8^+$ cells and of pro-inflammatory Th17 cells is increased in COVID-19 patients, and may be partly responsible for tissue damage in patients with severe disease [51,52]. Thus, agents that can counter the adaptive immune system pro-inflammatory response may be of value to COVID-19 patients. A number of studies have concluded that AT_2R are not expressed at high levels on cells of the adaptive immune system [87,88]. However, T cells and NK cells do express AT_2R at low levels [89,90], and increased levels of AT_2R-expressing T cells of $CD4^+$ and $CD8^+$ varieties appear to become important under certain disease conditions with a strong pro-inflammatory component. For example, $CD4^+$ AT_2R^+ $FoxP3^+$ were identified as a novel T-regulatory cell subset that was up-regulated in a rat model of myocardial infarction (MI) and in human heart failure patients, and acted anti-inflammatory via the secretion of IL-10 [91]. Myocardial transplantation of these $CD4^+$ AT_2R^+ $FoxP3^+$ T cells decreased infarct size and improved cardiac function in the rat MI model [91]. A similar study identified a cardioprotective $CD8^+$ $AT2R^+$ T-cell population, which increased during ischaemic heart injury. These cells secreted IL-10 upon AT_2R stimulation by C21, exhibited decreased IL-2 and IFN-γ expression, and contributed to maintaining viability of cardiomyocytes [92]. The protective- and anti-inflammatory effects of AT_2R-expressing $CD4^+$ T cells were also apparent in thoracic aortic aneurysm (TAA) [93]. Further studies which utilised naïve T cells isolated from mouse spleen and lymph nodes demonstrated that AT_2R stimulation with C21 under polarising conditions modified their differentiation, resulting in a less pronounced pro-inflammatory phenotype as indicated by lowered expression of IFN-γ and IL-17 and increased expression of the marker of regulatory T cells, FoxP3 [94]. Despite these studies that demonstrate protective, anti-inflammatory actions of AT_2R-expressing T cells, one study indicates the opposite. Namely, Caillon et al. [95] demonstrated that AT_2R-activation of Th17 cells induced secretion of the pro-inflammatory cytokine IL-17 that drove flow-mediated outward arterial remodelling, and proposed that this is necessary for collateral artery growth in ischaemic disease – once again, a beneficial action of AT_2R activation, but opposite in terms of inflammatory response. As stated above, AT_2R are also expressed on NK cells (particularly uterine NK cells) [89,96]. However, their specific roles in terms of influencing the immune system are unknown.

Thus, there is evidence that points to *direct* beneficial actions of AT_2R activation via cells of the innate and adaptive immune systems, which could be beneficial during the hyperinflammatory state in COVID-19. However, based on the available evidence the greater effect of AT_2R on the immune system is via actions at non-immune tissues and cells.

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Anti-inflammatory AT_2R actions via non-immune cells and tissues

There is a host of data indicating powerful anti-inflammatory actions of AT_2R within the parenchymal tissues of multiple organ systems throughout the body [73,75]. This includes the lungs [26,82,83,97], heart [85,92,98,99], kidney [100–103], vasculature [72,73,102,104–108] and the central nervous system (CNS) [109–116]. These anti-inflammatory actions of AT_2R activation at non-immune tissues and cells largely involve interruption of intracellular signalling pathways that lead to production of pro-inflammatory cytokines, and also through induction of pathways that elicit production of anti-inflammatory cytokines such as IL-10 [73,75,116]. The cellular and intracellular actions of AT_2R have been recently reviewed in detail elsewhere [75,117], and the objective of the current article is not to re-review them here. Rather, we have utilised a diagram (Figure 3) to illustrate and summarise the anti-inflammatory actions of AT_2R at various tissues/cells, and have included the intracellular signalling pathways that are likely of importance in these actions. Here, we have emphasised those anti-inflammatory effects that would likely be important for helping to counter the deleterious overactivation of pro-inflammatory mechanisms in COVID-19 patients, in particular those that subsequently lead to tissue fibrosis. Also included in the diagram are vascular and CNS actions of AT_2R, that are discussed in more detail in subsequent sections of this review.

As shown in Figure 3, literature evidence points to several intracellular steps that lead to a reduction in the production of pro-inflammatory cytokines following AT_2R stimulation [117]. In brief, agonist-induced AT_2R stimulation results in activation of tyrosine and serine/threonine phosphatases via an inhibitory G-protein [118–120]. In turn, these phosphatases make less nuclear factor-κB (NF-κB) available for translocation to the nucleus, and inhibit extracellular signal-regulated kinase 1/2 (Erk 1/2) and Janus kinase (Jak)/signal transducer and activator of transcription (Stat) pathways through dephosphorylation events [72,120]. The ultimate result is less production of pro-inflammatory cytokines. On the other hand, AT_2R stimulation of tyrosine and serine/threonine phosphatases and of Rac-α serine-threonine kinase (Akt) elicits activation of endothelial nitric oxide synthase (eNOS) and generation of nitric oxide (NO) and cyclic guanosine monophosphate (cGMP), the latter exerting anti-inflammatory effects [121]. While the mechanisms of AT_2R-induced IL-10 production are not established, it has been demonstrated that they require production of NO [102].

In summary, it is clear that activation of AT_2R exerts potent anti-inflammatory effects, via direct actions on immune system cells and non-immune tissues. Considering the dysregulated immune system and cytokine-storm observed in COVID-19 disease, selective AT_2R agonists may represent a viable approach for alleviating this excessive pro-inflammatory situation in COVID-19 patients, and in doing so help to reduce the development of downstream heart, kidney and lung complications (Figure 2).

Endothelial protection and anti-coagulation

Quite early during the pandemic it became clear that COVID-19 patients die not only from viral pneumonia causing ARDS and hypoxic respiratory failure, but also from cardiovascular complications, thrombotic events and multi-organ failure [122,123]. A unifying cause for these quite different pathologies may originate from the vascular endothelium [7–9,124].

Generally, there are two potential causes for endotheliopathy in COVID-19: either direct viral infection of endothelial cells or the so-called cytokine storm as described earlier in this review. Evidence indeed exists for the presence of both pathomechanisms in COVID-19-associated endotheliitis. Direct viral infection was demonstrated by Varga et al. and Ackermann et al. in endothelial cells from various vascular beds in biopsies from patients with a severe course of COVID-19 [10,125]. Regarding endothelial cell injury during COVID-19-induced cytokine storm, it is generally known, that increased plasma levels of cytokines cause direct cell injury by binding to their respective membrane receptors and initiating pro-inflammatory signalling cascades and eventually cell apoptosis [55,126,127]. Moreover, cytokines promote recruitment of cytotoxic leucocytes by increasing expression of adhesion molecules on the surface of endothelial cells [128].

Regardless of the origin of the pathological changes in endothelial cells in COVID-19, injury of these cells leads to their dysfunction, which is characterised by a shift of the vascular equilibrium towards more vasoconstriction, loss of the anti-coagulant state, endothelial cell death and barrier-breakdown with subsequent tissue oedema and facilitated infiltration of inflammatory cells [8,9]. In addition, inhibition of ACE2 enzymatic activity in COVID-19 prevents degradation of bradykinin by ACE2 thus promoting bradykinin-mediated vascular leakage [129]. These changes contribute to the development of ARDS, to impaired function of other organs such as kidney, heart or brain and to thromboembolic complications [8,9].

Several clinical studies have indeed reported an increase in markers of endothelial dysfunction and coagulation indices [e.g. von Willebrand factor (VWF) antigen, VWF activity, factor VIII activity, D-dimers, fibrinogen, fibrin

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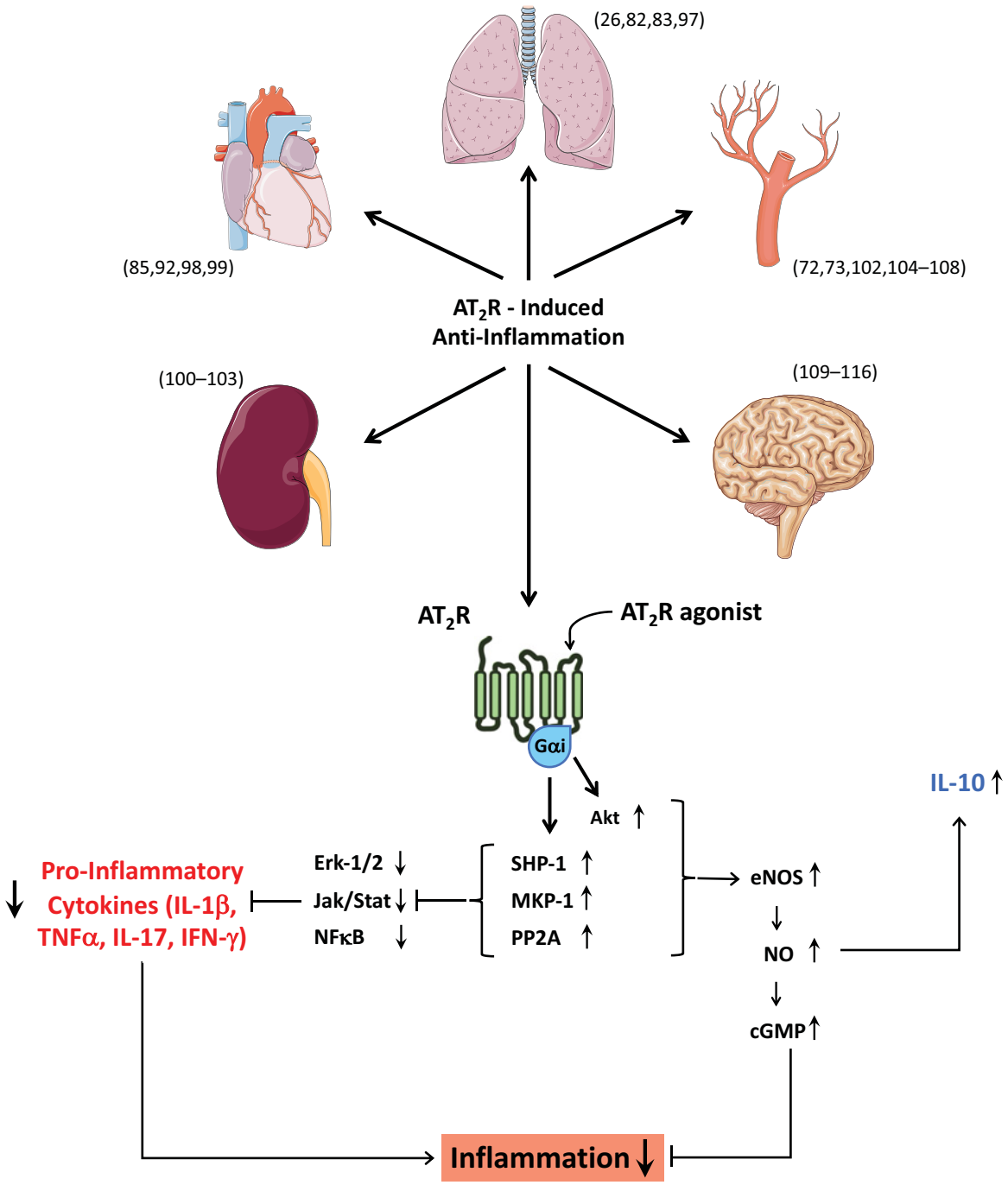

Figure 3. Anti-inflammatory effects of AT2R activation at organ and intracellular levels

This diagram illustrates the anti-inflammatory effects that have been described following agonist-induced AT_2R activation, at both the whole organ and intracellular levels. Numbers in parentheses next to the organs are reference numbers – see References list. Abbreviations: Akt, Rac-α serine-threonine kinase; AT_2R, angiotensin AT_2-receptor; cGMP, cyclic guanosine monophosphate; eNOS, endothelial nitric oxide synthase; ERK1/2, extracellular signal-regulated kinase 1/2; MKP-1, mitogen-activated protein kinase phosphatase 1; NF-κB, nuclear factor κB; NO, nitric oxide; PP2A, protein phosphatase 2A; SHP-1, Src homology region 2 domain-containing phosphatase-1; Stat, signal transducer and activator of transcription. Organ pictures are reproduced from Servier Medical Art Commons Attribution 3.0 Unported License (http://smart.servier.com). Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License.

degradation product; prolonged prothrombin time and partial thromboplastin time], in patients with COVID-19 [7,122,130]. Importantly, several of these markers correlated positively with the prognosis for the individual patient [130]. Other studies reported microangiopathy, capillary microthrombi and disseminated intravascular coagulation by histological examination of post-mortem lungs from COVID-19 patients [125,131].

There is good experimental evidence to support the assumption that AT_2R-stimulation will act in a protective manner in the event of SARS-CoV2 induced endothelial injury, although data from preclinical or clinical studies in SARS-CoV2-infected individuals is yet to be obtained. The clinical trial with the AT_2R-agonist C21 in patients with COVID-19 (reviewed later in this article) will presumably provide such data [13].

Generally, AT_2R-stimulation promotes mechanisms which are beneficial for endothelial function such as activation of eNOS leading to NO generation or synthesis of the vasodilatory mediator epoxyeicosatrienoic acid from arachidonic acid [121,132,133].

Additionally – as reviewed above – stimulation of the AT_2R acts anti-inflammatory, which also applies to the vasculature. A study by Sampson et al. in human umbilical vein endothelial cells and in $ApoE^{-/-}$ mice fed a high-fat diet provided an in-depth examination of potential anti-inflammatory effects of AT_2R-stimulation by C21 in the vasculature and found an inhibition of adhesion molecule expression and monocyte adhesion *in vitro* and an attenuation of leucocyte adhesion *in vivo* [105]. The authors could further show an AT_2R-mediated inhibition of NF-κB activity resulting in reduced IL-6 and TNFα cytokine expression. This latter observation confirmed prior reports about an inhibition of the key regulator of cytokine transcription, NF-κB, by AT_2R-stimulation in rat fetal vascular smooth muscle cells and human dermal fibroblasts [72,134]. An anti-inflammatory effect in vasculature, i.e. a reduction in monocyte infiltration in aortas from deoxycorticosterone acetate-hypertensive rats, has also been shown for the ACE2-dependent, endogenous AT_2R-agonist Ang-1-9 [135]. Notably, both studies identified AT_2R-mediated, anti-inflammatory effects, which specifically counteracted pro-inflammatory mechanisms relevant for COVID-19-associated endothelial injury.

As stated above, endothelial barrier breakdown and increased vascular permeability are important factors contributing to tissue oedema, which is a main reason for impaired gas exchange in ARDS [136]. By measuring hydraulic permeability in rat mesenteric venules, Ereso et al. could show that AT_2R-stimulation by the selective agonist CGP42112A or by Ang II under concomitant AT_1R-blockade attenuated microvascular fluid leakage, which had been induced by platelet activating factor [137]. Further evidence for a reduction in vascular leakage by AT_2R-stimulation comes from a stroke model in mice, in which treatment with C21 decreased blood–brain barrier (BBB) permeability and subsequent development of cerebral oedema [111].

Finally, a few studies have suggested that the AT_2R has anti-thrombotic effects, which could help to counteract the pro-coagulant state in COVID-19. Chabielska et al. described that the AT_1R-blocker Losartan significantly reduced thrombus weight in a model of vena cava thrombosis in Wistar rats with two-kidney, one-clip induced renal hypertension [138]. The effect of Losartan was blocked by the AT_2R-antagonist PD123319 indicating that indirect AT_2R-stimulation by elevated levels of Ang II acting on the unopposed AT_2R was the underlying mechanism of the Losartan effect.

Interestingly in the context of COVID-19 induced hypercoagulability, a study by Balia et al. found an inhibitory effect of AT_2R-stimulation on LPS-induced tissue factor expression in peripheral blood mononuclear cells [139]. Apart from mononuclear cells, tissue factor is also highly expressed by vascular, subendothelial cells and is essential for the initiation of the clotting cascade in response to inflammation or tissue injury [140]. Tissue factor on monocytes, which is increased by elevated cytokine levels and also by Ang II, plays a key role in triggering disseminated intravascular coagulation and thrombotic microangiopathy in bacterial or viral sepsis [140]. Increased tissue factor expression on monocytes has also been shown for patients with a severe course of COVID-19, and expression levels correlated with severity of disease [141]. Consequently, experimental data by Balia et al. showing an AT_2R-mediated reduction in tissue factor expression on monocytes points to a potential therapeutic effect of AT_2R-agonists in COVID-19 related coagulopathy, which, however, still needs to be proven in clinical studies [139].

Anti-fibrotic effects of AT2R activation

It is established that pulmonary inflammation is a major causative factor in the development of pulmonary fibrosis [142,143]. As indicated in Figure 2 one of the major long-term outcomes in COVID-19 is the development of pulmonary fibrosis [66–69], likely due to the hyperinflammatory state. In fact, pulmonary fibrosis is one of the pathological situations in which AT_2R agonists may have beneficial effects for COVID-19 patients. This assumption is based on several preclinical studies in which AT_2R-agonists were proven to have profound anti-fibrotic activity within many disease states, including pulmonary-, cardiac-, renal-, aortic and pancreatic fibroses. These anti-fibrotic actions have

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recently been extensively reviewed [117,144], and so the description of all of these actions will not be recapitulated here. Rather, we focus on the pulmonary anti-fibrotic actions, that are likely of direct importance to patients with COVID-19 disease.

Two preclinical studies have demonstrated that the AT_2R agonist C21 prevents and reverses pulmonary fibrosis. In the first, daily systemic treatment of rats with C21 beginning 2 weeks after induction of pulmonary fibrosis with monocrotaline reversed interstitial and perivascular fibrosis [26]. In addition, C21 treatment decreased transforming growth factor-β mRNA expression, which was greatly increased in the monocrotaline-treated rats [26]. In the second study from the same group, pulmonary fibrosis was induced in rats by intra-tracheal administration of bleomycin and systemic C21 administration began either at the same time as bleomycin (prevention) or 3 days later (treatment); in both cases, C21 administration continued on a daily basis for 2 weeks. In the prevention paradigm, C21 significantly attenuated the formation of pulmonary fibrosis and in the treatment paradigm, fibrosis progression was almost completely stopped from the start of the day of the treatment [82]. Associated markers of fibrosis, such as collagens 1 and 3, connective tissue growth factor, IL-13, and tissue inhibitor of matrix-metalloproteinases were all reduced by C21-treatment [82].

The importance of these findings is manifold: they not only proved the principle that AT_2R activation by an agonist is beneficial in pulmonary fibrosis, they also laid the groundwork for the development of C21 as a potential therapeutic for idiopathic pulmonary fibrosis [145]. In addition to the above described anti-fibrotic actions, the attenuating effects of AT_2R agonists on pulmonary inflammation will presumably have a preventive effect with regard to the development of pulmonary fibrosis in patients with a severe, hyperinflammatory courses of COVID-19 [26,82,97]. However, this assumption will have to be proven in future clinical studies. The ongoing trial with C21 in patients with COVID is most likely too short (7 days treatment) and does not include enough patients to detect any potential effects of C21 on the development of pulmonary fibrosis [13].

CNS effects of AT_2R activation

The most common neurological complications of severe COVID-19 are encephalitis and ischaemic stroke [146,147].

Ischaemic stroke associated with COVID-19 disease occurs in patients of all ages, with more severe strokes, worse functional outcomes and higher mortality rates than are seen in a comparable group of non-COVID-19 patients [148–152]. Thus far, it appears that the major causes for these ischaemic strokes are derived from the hyperinflammatory state and resulting coagulopathy observed in COVID-19 patients, which lead to thromboembolism [153], neurovascular endothelial dysfunction, BBB breakdown, and consequent generalised activation of the CNS innate immune system [149]. Cardiomyopathy, and consequent intracardiac formation of thrombi, which may travel to the brain, is another potential cause of ischaemic stroke in COVID-19 [153].

AT_2R agonists appear well-positioned to be of significant benefit to COVID-19 patients who have developed ischaemic strokes. Preclinical studies in rats and mice from multiple independent investigative teams, using several different models of ischaemic stroke, have demonstrated the efficacy of AT_2R agonists applied post-stroke in reducing the severity of intracerebral infarcts, and improving behavioural/neurological outcomes [110–112,115,154–158]. Details of these studies and the mechanisms of neuroprotection have been reviewed by us, recently [159]. The factor that might make AT_2R agonists a viable approach for ischaemic stroke in COVID-19 patients is their multiple modes of action against stroke that include neuroprotective, vascular and regenerative aspects [159]. The neuroprotective aspects include anti-inflammatory, anti-apoptotic and anti-oxidant effects [159]. Thus, in COVID-19 induced strokes AT_2R agonist-induced anti-inflammatory and anti-oxidant effects might not only help to depress the cytokine storm, alleviate neurovascular endothelial dysfunction and coagulation, but also suppress the generalised activation of the innate CNS immune system. A further mode of action of AT_2R agonists in ischaemic stroke is restoring BBB integrity – a clear benefit in severe pro-inflammatory conditions where the BBB is compromised [111]. A final important aspect is that our group has demonstrated that an AT_2R agonist (C21) can be applied directly to the brain via an intranasal (nose to brain) route, where it exerted significant protective effects against ischaemic stroke in a rodent model [157]. Such a delivery system can easily be replicated in humans.

To summarise, based upon preclinical findings there is strong potential that AT_2R agonists may be effective in at least alleviating the severity of ischaemic strokes in COVID-19 patients. The caveat is that thus far, there has been no clinical evaluation of the actions of AT_2R agonists in human stroke patients.

Clinical trial of C21 in patients with COVID-19

The non-peptide, orally active AT_2R-agonist C21 (Vicore Pharma, Gothenburg, Sweden; now also termed as VP01) has been tested for safety and efficacy in a Phase II clinical trial in patients with COVID-19, the so called ATTRACT

Table 1 Inclusion and exclusion criteria for the ATTRACT trial

Inclusion criteria	Exclusion criteria
Written informed consent, consistent with ICH-GCP R2 and local laws, obtained before the initiation of any trial related procedure	Any previous experimental treatment for COVID-19
Diagnosis of coronavirus (SARS-CoV)-2 infection confirmed by polymerase chain reaction (PCR) test < 4 days before Visit 1 with signs of an acute respiratory infection	Need for mechanical invasive or non-invasive ventilation
Age: >18 and <70 years	Concurrent respiratory disease such as COPD (chronic obstructive pulmonary disease), IPF and/or intermittent, persistent or more severe asthma requiring daily therapy or any subjects that have had an asthma flare requiring corticosteroids in the 4 weeks (28 days) prior to COVID-19 diagnosis
CRP >50 and <150 mg/l	Participation in any other interventional trial within 3 months prior to Visit 1
Admitted to a hospital or controlled facility (home quarantine is not sufficient)	Any of the following findings at Visit 1: - Positive results for hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (HCVAb) or human immunodeficiency virus 1+2 antigen/antibody (HIV 1+2 Ag/Ab - Positive pregnancy test
In the opinion of the Investigator, the subject will be able to comply with the requirements of the protocol	Clinically significant abnormal laboratory value at Visit 1 indicating a potential risk for the subject if enrolled in the trial as evaluated by the Investigator
Males and females	Concurrent serious medical condition with special attention to cardiac or ophthalmic conditions (e.g. contraindications to cataract surgery), which in the opinion of the Investigator makes the subject inappropriate for this trial
	Malignancy within the past 3 years with the exception of *in situ* removal of basal cell carcinoma and cervical intraepithelial neoplasia grade I
	Treatment with any of the medications listed below within 1 week prior to Visit 1: - Strong Cytochrome p450 (CYP) 3A4 inducers (e.g. rifampicin, phenytoin, St. John's Wort, phenobarbital, rifabutin, carbamazepine, anti- HIV drugs, barbiturates) - Warfarin
	Pregnant or breast-feeding female subjects
	Female subjects of childbearing potential not willing to use contraceptive methods as described in detail in the study protocol
	Male subjects not willing to use contraceptive methods as described in detail in the study protocol
	Subjects known or suspected of not being able to comply with this trial protocol (e.g. due to alcoholism, drug dependency or psychological disorder)

trial (https://www.clinicaltrials.gov/ct2/show/NCT04452435 and https://vicorepharma.com/the-attract-study/) [13]. The trial was completed on 13 October 2020 (final data collection date for primary outcome measure), but has not yet reported any results (status on 13 November 2020). ATTRACT is a randomised, triple-blind, placebo-controlled trial with parallel design, for which 106 patients were enrolled and randomised to receive either placebo or C21 (100 mg twice daily) po. Treatment duration was 7 days, and C21 or placebo were added on top of standard of care medication. With regard to inclusion and exclusion criteria, it is important to note that only a specific subgroup of patients diagnosed for COVID-19 was eligible for recruitment, which were patients hospitalised for COVID-19 associated, respiratory symptoms, but not requiring intensive care or mechanical invasive or non-invasive ventilation. This specific subgroup of patients was most likely selected for targeting patients with a developing or already existing hyperinflammatory response, but not yet suffering from life-threatening complications. Primary outcome was defined as the change in C-reactive protein (CRP) from treatment start to the end of the 7-day treatment period, i.e. the trial has mainly tested, whether AT_2R-stimulation is able to attenuate the inflammatory reaction in COVID-19 and prevent or milden the cytokine storm. More detailed inclusion and exclusion criteria can be found in Table 1. The study was performed as a multi-centre trial located in the U.K. and India. ATTRACT is expected to report in late 2020.

Conclusions

The COVID-19 pandemic that is a result of infection with the SARS-CoV-2 coronavirus is affecting millions of people and the death toll has now exceeded 1.3 million victims. It is one of the major challenges in medical research of our time and one of the biggest unmet medical needs. In an enormous effort, scientists all over the world are trying to develop vaccines and effective treatments to bring down the number of COVID-19 associated fatalities and severe courses of disease and to get us all back to a life with normal social interactions and daily routines.

One approach in the search for a treatment of COVID-19 is to find drugs that counteract the imbalance of the RAS in this disease, which is caused by loss of enzymatic activity of ACE2, the enzyme which serves as the binding site for

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cellular entry of SARS-CoV-2. The result of SARS-CoV-2/ACE2 binding is that there is a weakening of the protective arm of the RAS and an overactivation of the classical, AT_1R-dependent arm of the RAS.

Based on a multitude of preclinical studies, which are reviewed in this article, we propose, that stimulation of the angiotensin AT_2R with respective agonists may be a promising approach for the treatment of COVID-19. This is for two main reasons, one of which is a compensation for the loss of endogenous, ACE2-mediated synthesis of AT_2R- and Mas-agonists by application of an exogenous, synthetic AT_2R-agonist. Due to an intense cross-talk between the AT_2R- and the Mas-dependent protective arms of the RAS, it can be assumed that application of an AT_2R-agonist will also enhance signalling of and protection by Mas. Second, preclinical evidence points to a therapeutic and protective effect of AT_2R-agonists in many COVID-19 associated pathologies such as inflammation (cytokine storm), lung injury and fibrosis, endotheliitis, coagulopathy and stroke.

Our theoretical considerations regarding a rationale for AT_2R-agonists for the treatment of COVID-19 may soon be proven right or wrong, since the non-peptide AT_2R-agonist C21 has been tested in a Phase II clinical trial in patients with moderately severe COVID-19 (with hospitalisation but no ICU treatment). The study will presumably report first results by the end of 2020.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contribution

U.M.S. and C.S. wrote and critically revised the manuscript.

Ethics Approval

Not applicable. This is a review of published studies.

Abbreviations

ACE, angiotensin converting enzyme; ACE2, angiotensin converting enzyme 2; ALI, acute lung injury; Ang-(1-7), angiotensin-(1-7); Ang-(1-9), angiotensin-(1-9); Ang II, angiotensin II; Ang III, angiotensin III; ARB, AT_1 receptor blocker; ARDS, acute respiratory distress syndrome; AT_1R, angiotensin AT_1-receptor; AT_2R, angiotensin AT_2-receptor; ARDS, acute respiratory distress syndrome; ATTRACT, **A**ngiotensin II **T**ype **T**wo **R**eceptor **A**gonist in **C**OVID-19 **T**rial; BBB, blood–brain barrier; CNS, central nervous system; C21, Compound 21; COVID-19, corona virus disease 2019; cGMP, cyclic guanosine monophosphate; ELISA, enzyme-linked immunosorbent assay; eNOS, endothelial nitric oxide synthase; IFN-γ, interferon-γ; IL, interleukin; Mas, receptor Mas; MrgD, Mas-related G-protein coupled receptor member D; NK, natural killer; NF-κB, nuclear factor κB; NO, nitric oxide; RAS, renin–angiotensin system; SARS-CoV, severe acute respiratory syndrome coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TNFα, tumour necrosis factor α; TLR, Toll-like receptor; TMPRSS2, transmembrane protease serine 2.

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