Strengthening CoViD-19 therapy via combinations of RAS modulators

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ABSTRACT

Evidence has accumulated that the pathology of CoViD-19 is strongly related to the renin-angiotensin system (RAS). The blockage of the angiotensin converting enzyme 2 (ACE2) by the SARS-CoV-2 virus leads to downstream consequences such as increased vascular tone, extensive fibrosis and pronounced immune reactions. Different approaches to tackle the adverse viral effects by compensating the lost ACE2 function have been suggested. Here, we use an unequal-arm lever model to describe a simplified version of the biased regulation exercised by the angiotensin II and angiotensin-(1–7) hormones, which are the substrate and the product of ACE2, respectively. We reason upon the lever dynamics and its disruptions caused by the virus, and propose that a combination of RAS modulators will most efficiently compensate the imbalance due to the excess of angiotensin II and the scarcity of angiotensin-(1–7). Specifically, we focus on the possible benefits of the simultaneous application of two agents, a MAS-receptor agonist and an angiotensin-II-type-2-receptor agonist. We conjecture that this combination has the potential to introduce a beneficial synergistic action that promotes anti-hypoxic, anti-fibrotic and anti-proliferative effects, thereby improving the clinical management of acute and chronic CoViD-19 pathologies.

Introduction

The renin-angiotensin system (RAS, Fig. 1) is a complex network of molecules that regulates cell growth, differentiation and proliferation, fluid and electrolyte balance, and vascular tone, and it is critical for inflammatory responses in many organs such as kidneys, liver and lung, and in the cardiovascular system [1,2].

Renin is an enzyme that converts the protein angiotensinogen to angiotensin I (Ang I), a decapeptide [2]. Ang I then binds the angiotensin converting enzyme (ACE), which processes it into angiotensin II (Ang II), an octapeptide [1,2]. In turn, Ang II interacts with two types of receptors, Ang II type 1 (AT_1) and Ang II type 2 (AT_2) with opposing effects: increasing cell proliferation, inflammatory reactivity and vascular tone via the AT_1 receptor, and mitigating these same processes via the AT_2 [2,3]. Ang II is also processed by the angiotensin converting enzyme 2 (ACE2) into angiotensin-(1–7) (Ang-(1–7)), a heptapeptide that binds

the MAS receptor causing vasodilation and having anti-inflammatory and anti-proliferative effects [1,4]. The network of RAS interactions includes other molecules beyond those mentioned here; however the core participants needed for a minimal model that still captures the main regulatory capabilities of the system are the ones described above.

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) affects RAS because it uses ACE2 as cell entry point [1,5,6]. It infects mainly ciliated respiratory epithelial cells and type-II pneumocytes that express ACE2, thus causing the lung to be the main affected organ [7]. In addition, the virus has a tropism for vascular endothelial cells, which also express ACE2, leading to cerebrovascular, cardiac and renal involvement [7]. The infected cells are attacked by the immune system attempting to clean the virus [7], leading to a decrease in ACE2 concentration and to the disruption of the normal functioning of RAS.

Fig. 1. Main RAS participants and interactions.

Fig. 2. A lever schematic of the biased regulation by the antagonistic couple of hormones Ang II / Ang-(1–7) (a) and their disturbance by SARS-CoV-2 (b).

Hypothesis

In what follows, we build a simplified model of the opposing action of the Ang II/Ang-(1-7) hormones, which are represented as a lever with unequal arms. This is a qualitative approach [8,9,10] that nonetheless captures a crucial aspect of RAS activity, namely its pronounced bias to avoid regimes that are highly dangerous on the short term. We then use this model to formulate a hypothesis about how this biased regulation is disturbed by SARS-CoV-2, leading to new strategies to devise treatment. Consequently, we propose to test our hypothesis by evaluating the therapeutic effectiveness of a combination of RAS modulators that should most efficiently compensate the disturbances inflicted by the virus.

To maintain processes within the physiological limits, some regulatory systems, including RAS, exert unequal influences along two opposing directions of change, in a similar fashion to the workings of an unequal-arm lever [8,9,10]. Powerful mechanisms are installed to prevent deviations that may quickly lead to death. However, this happens at the expense of a relatively higher tolerance to deviations in the other direction. For example, severe drops in blood pressure have a significant short-term mortality, and therefore many means exist to ensure against them. As a result, there is a permissiveness for higher blood pressure values and an overwhelming relative occurrence of chronic hypertensive conditions versus chronic hypotensive ones whose occurrence is virtually null.

In our case, the regulated parameters are vascular tone, cell proliferation and immune reactivity, while the hormones affecting them are Ang II and Ang-(1–7). Ang-(1–7), the product of ACE2, is the main force leading to an upward movement of the lever arm and a corresponding downward shift of the parameters. Ang II, the substrate of ACE2 is the principal force behind their increases, causing the opposite motion. Since low vascular tone, and low rates of cell proliferation and inflammatory reactions are more deleterious in the short term, Ang-(1–7) is given a shorter lever arm, whereas Ang II operates on a longer one (Fig. 2a). Effectively blocking ACE2, SARS-CoV-2 causes both an excess of Ang II and a scarcity of Ang-(1–7), and tips the balance of the already biased system in a twofold way, removing force from the short lever arm and adding force to the long one (Fig. 2b), thus leading to catastrophic increases of the regulated parameters.
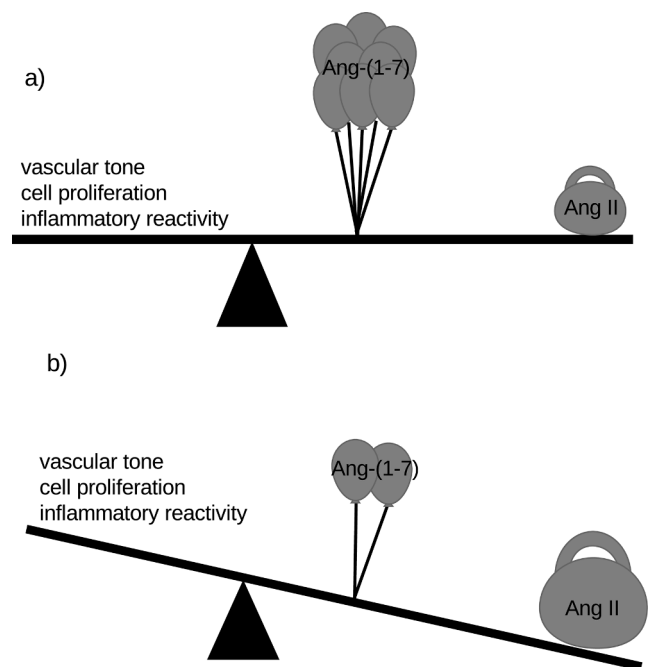
We note that our hypothesis is naturally consistent with observations about CoViD-19 pathology. In fact, age has emerged as the leading risk factor for developing severe CoViD-19 symptoms [7]. Previous results have correlated the severity of acute lung injuries with the age-dependent reduction of the ratio ACE2/ACE [2]. Based on this, we surmise that the lower ACE2/ACE ratio in elderly CoViD-19 patients results in a relatively smaller number of ACE2 molecules that can escape the viral blockade and continue converting Ang II to Ang-(1–7). In turn, this causes an even more pronounced overstimulation of the long lever arm and a harsher action deficit of the short one compared to young patients, in agreement with our model and offering a strong indication of the importance of the ACE2 viral blockade.

The proliferative and immune processes considered above are indeed particularly undesired consequences of the ACE2 blockade. Ang II leads to recruitment of inflammatory cells, adhesion of monocytes and neutrophils to endothelial and mesanglial cells, and expression, synthesis and release of cytokines and chemokines, whereas Ang-(1–7) negatively modulates leucocyte migration, cytokine expression and release, and fibrogenic pathways [11]. However, the immune signature of SARS-CoV-2 itself consists of impaired interferon responses, elevated serum cytokines and lymphopenia [7]. Thus, a SARS-CoV-2 infection has a twofold pro-inflammatory effect, mediated at the same time by the native immune response and by the disruption of the RAS balance. In addition, these inflammatory mechanisms are likely to have deleterious synergistic effects. For example, studies on viral pneumonia of influenza origin uncovered that direct cytotoxic effects are not the only drivers of mortality, as a major contribution comes from the host's inability to dampen inflammation and to repair damaged tissue [7]. Therefore, we believe that the severity of the two main problematic conditions in CoViD-19 management, namely the pneumonia-induced acute respiratory distress symptom (ARDS) [7] and the pulmonary fibrosis in CoViD-19 survivors [12], is directly affected by the RAS involvment. Consequently, we conjecture that restoring RAS balance will enormously improve both the acute organ damage and the post-infection tissue repair processes.

From the point of view of lever dynamics, one possible strategy to offset the virus-related RAS disturbance is to alleviate pressure on the

long arm. This can be achieved by decreasing Ang II production. ACE inhibitors have been proposed to this effect [1]. Many CoViD-19 patients already use them routinely for myocardial infarction, heart failure, cerebrovascular and chronic kidney disease [13]. Studies showed no positive correlation between severity of CoViD-19 and use of ACE inhibitors, and reported benefits to the hypertensive cohort [13]. However, other enzymes insensitive to these compounds are able to take over production of Ang II [5], which might limit efficacy. Additionally, this approach may be inherently impaired in restoring RAS balance, as it would cause a downstream decrease of the already lowered Ang-(1–7).

An alternative strategy aimed at relieving the long lever arm could be to block the AT_1 receptors, rather than blocking the production of Ang II. The selectivity provided by the current AT_1-receptor blockers [1,3] would preserve the protective effects via the AT_2 receptors. Many CoViD-19 patients already undergo treatment with AT_1-receptor blockers for pre-existing disorders. Similarly to ACE inhibitors, evidence shows no positive correlation with disease severity, and beneficial effects in the hypertensive cohort [13]. However, the introduction of ACE inhibitors and/or AT_1 receptor blockers in CoViD-19 patients that are not already receiving them requires further investigation.

These considerations show that influencing the long lever arm is not straightforward, as it is yet unclear whether ACE inhibitors and AT_1-receptor blockers are applicable to patients without other pathologies that already necessitate their use. Accounting for this and for the bias against the counterpart short arm, we propose that additions to CoViD-19 therapy aimed at RAS management should focus on compensating the loss of the short-arm effects. Ang-(1–7) directly opposes Ang II using the short arm. Therefore, to restore balance, one could supplement the peptide itself or a suitable receptor agonist. Ang-(1–7) peptide and MAS-receptor agonists have been suggested as additions to CoViD-19 therapy [1,5,14]. The linear peptide is rapidly metabolized and non-specific in high doses [15]. Cyclic Ang-(1–7) (cAng-(1–7)) is a better option, as it does not present these shortcomings and has shown protective effects for the endothelium when tested on models of myocardial infarction [15]. The non-peptide agonist AVE0991 has demonstrated promising results when tested on models of inflammatory and hypoxic injury such as kidney and cerebral ischaemia, and antigen-induced arthritis, modulating inflammation and fibrosis and decreasing pro-inflammatory cytokines, neutrophil influx, and leucocyte rolling and adhesion [11]. However, further specific studies on lung pathologies are needed to assess its organ-protective effects against inflammation and fibrosis.

Within the framework of our model, it is critical to strengthen the effects of the short lever arm via a second modulator, to overcome the natural bias against them. The ultimate candidates for this purpose are AT_2-receptor agonists, as AT_2-receptor activation causes the desired decreases in the disregulated parameters. The natural agonist is Ang II itself, probably as a precaution against its overwhelming strength mediated by the AT_1 receptors. Its effects via the AT_2 pathway are much more subtle, likely due to the low receptor expression in adults [16]. However, AT_2 receptors notably re-express during vascular injuries [16]. Since vasculopathy is characteristic of CoViD-19 [7], AT_2 receptors are likely re-expressed in the affected tissues, which would concentrate the agonist activity where it's most needed.

Compound 21 (C21) is a non-peptide agonist highly selective for AT_2 receptors [16]. It has shown great organ-protective and anti-fibrotic effects: in models of myocardial infarction, its application leads to decreased scarring and reduced expression of cytokines in peri-infarct tissue [16], and it also reduces kidney inflammation and fibrosis in models of hypertension [17]. Most importantly, in models of pulmonary hypertension and cardiopulmonary fibrosis, it reverses lung fibrosis and prevents right ventricular fibrosis [2,18], making it a stellar candidate for a second modulator to restore RAS balance.

Note that our underlying assumption is that the effects of the two types of agonists are additive. We believe this is a reasonable expectation to hold, given existing experimental evidence. In particular, studies have shown that a combination of Ang-(1–7) with an ACE inhibitor was more efficient in attenuating diabetic cardiac fibrosis than either agent alone [19], and that cAng-(1–7) had add-on effects to an ACE inhibitor (lisinopril) in the treatment of experimental diabetic nephropathy [20]. Moreover, the synergistic action of a MAS and an AT_2 receptor agonist has been reported in relation to heart pathology, in models of ischaemic myocardial injury where the combined application of C21 and AVE0991 leads to lower infarct size [21]. Nonetheless, we do believe that combinations of this kind merits further testing, especially in models of inflammatory lung injury and lung repair.

Conclusions

SARS-CoV-2 disrupts the functioning of ACE2, a main RAS component, leading to accumulation of its substrate, Ang II, and depletion of its product, Ang-(1–7). The two hormones normally have opposing effects on inflammation, proliferation and vascular tone, with the Ang-(1–7) lowering activity being biased against in healthy individuals. We believe the indirect stimulation of proliferation and inflammation by SARS-CoV-2 adds to the complex immune signature of the virus, and worsens the tissue damage caused by the inflammatory immune response to the infection itself. These considerations make RAS a promising target to increase the effectiveness of CoViD-19 therapy.

To model the action of the Ang II/Ang-(1–7) system, we represent it as an unequal-arm lever whose short arm is critically incapacitated during CoViD-19. From it, we conjecture that a combinations of modulators, namely a MAS agonist and an AT_2-receptor agonist, will have beneficial additive anti-fibrotic and immune-modulating activities. We propose that our hypothesis about the mutually potentiating effects of such a combination be tested by applying a MAS and a AT_2 agonist to experimental models of acute lung injury and lung repair. Positive confirmation of our expectations has the potential to lead to substantial increase of the therapeutic success of ARDS and improvement of the management of the long term effects of CoViD-19.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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