Countermeasure and therapeutic: A(1–7) to treat acute respiratory distress syndrome due to COVID-19 infection

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Abstract
In the wake of the COVID-19 pandemic it has become clear that there is a need for therapies that are capable of reducing damage caused to patients from infections. Infections that induce Acute Respiratory Distress Syndrome (ARDS) are especially devastating because lung damage is so critical and difficult to manage. Angiotensin (1–7) [A(1–7)] has already been shown to protect pulmonary health and architecture in various models of disease. There is also evidence that A(1–7) can modulate immune function and protect various organs (lung, kidney, and heart) from oxidative damage and inflammation. Here we focus on making a case for the development of novel therapies that target the protective arm of the Renin Angiotensin System (RAS).

Keywords
COVID, ARDS, respiratory infection, angiotensin (1–7), ACE2

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SARS-CoV-2 infection. The pathologic hallmarks of human ALI/ARDS from any cause includes neutrophilic alveolitis, hyaline membranes secondary to protein transudation and precipitation in the airspace, microthrombi secondary to the generation of procoagulant mediators and endothelial injury, and epithelial and endothelial injury.

**Mechanistic overview of COVID-19 and the Renin Angiotensin System**

The Renin-Angiotensin System (RAS) can be thought of as a hormonal system with two axes—the ACE/Ang-II/AT1R axis (pathological arm) and the counter-regulatory ACE2/A(1–7)/Mas receptor axis (protective arm). The ACE/Ang-II/T1R axis has now been implicated in pulmonary, cardiovascular, renal and central nervous system pathophysiology.3 In vivo, Mas acts as a functional antagonist of the AT1R, thereby inhibiting the actions of A-II.4 A(1–7) is the endogenous ligand of the Mas receptor and a member of the protective RAS.

SARS-CoV-2, like SARS, binds to the cell surface protein receptor, ACE2, a member of the ACE2/A(1–7)/Mas axis. SARS pathology emerges, in part, due to the reduced ability of ACE2 to cleave Ang-II, a pro-inflammatory, fibrotic peptide, to A(1–7) (Figure 1).2 Ang-II is a potent vasoconstrictor that can increase lung injury and lung edema.3 A(1–7) counteracts the effects of angiotensin II and mobilizes endogenous regenerative processes.4 In SARS-infected animals, increased levels of Ang-II were observed due to reduced ACE2 activity. In this study ACE2 cleaves angiotensin II to A(1–7), thereby reducing the pathological activities of angiotensin II and increasing the protective arm of the RAS through the ACE2/A(1–7)/Mas axis.6 These data suggest the hypothesis that IV treatment with A(1–7) would reduce inflammation and oxidative stress, as well as rebalance the RAS. Moreover, the use of A(1–7) to combat SARS-CoV-2 is finding support in the peer-reviewed literature.7

**Clinical observations supporting a role for A(1–7)**

In a recent study of ICU patients with ARDS, increased serum levels of A(1–10) and reduced serum levels of A(1–7) at the time of admission, which indicates dysregulation of angiotensin peptide metabolism, were observed in those patients that did not survive.8 Importantly, patients with this degree of dysregulation of angiotensin peptide metabolism did not survive, despite aggressive ventilator-assisted pulmonary therapy. Further, a recent publication showed that circulating Ang-II levels increased with increased SARS-CoV-2 viral load and reduced Pa02/Fi02 levels.9

**Mechanistic studies supporting a role for A(1–7) in the treatment of ARDS**

Our proposed treatment of humans with COVID-19 induced ARDS by A(1–7) is also supported by animal models of ARDS (ventilator, oleic acid, and sepsis induced), pulmonary hypertension (PH), and fibrosis.10–17 Consistent across these studies is an imbalance between ACE/Ang II/AT1 and the ACE2/Ang-(1–7)/Mas axis of the RAS occurring in animals with lung disease.

In an experimental study published in Nature in 2005, Imai et al.6 showed that ACE2, which converts A-II to A(1–7) by cleavage of one amino acid, protects mice from ARDS induced by acid aspiration or sepsis. This is attributed to the fact that ACE2 will decrease A-II concentration and, thus, reduce the activation of the AT1R. A separate study by Shenoy et al.14 in rats showed positive effects in a pulmonary fibrosis model using lentiviral packaged A(1–7)-fusion genes or ACE2 cDNA. Overexpression of A(1–7) significantly prevented the associated negative effects of pulmonary fibrosis, namely: (1) increase in right ventricular systolic pressure and development of right ventricular hypertrophy; (2) excessive collagen deposition; (3) the decreased expression of ACE and ACE2; (4) increased pro-inflammatory cytokines; and (5) increased protein levels of the AT1R. Overexpression of ACE2 achieved similar protective effects. Blockade of the Mas receptor abolished the beneficial effects of A(1–7), confirming the role of the Mas receptor and A(1–7) in protection of the lung.

In vivo, A(1–7) protects against ventilator-induced ARDS in mice.15 Male C57/Bl6J mice were randomly assigned to three groups of five animals each. Animals in Group 1 (low V0) were continuously ventilated with a tidal volume (Vt) of 10mL/kg and a positive end-expiratory pressure of 2 mmHg.
In Group 2 (high Vt), ventilator-induced severe hypoxemia and pulmonary edema (ARDS) was induced by over-ventilation with tidal volumes of 20mL/kg and a positive end-expiratory pressure of 2mmHg. In Group 3 (high Vt + A(1–7)), VILI was induced as in Group 2, and infusion of Angiotensin 1–7 at 5 pmol/kg per minute was initiated with the start of high tidal volume ventilation. Over-ventilation with high tidal volumes of 20mL/kg caused ventilator-induced ARDS, evident as increased lung wet-to-dry weight ratio, decreased arterial oxygenation, and increased lung myeloperoxidase (MPO) activity. A(1–7) largely attenuated the development of ARDS, as demonstrated by a normalization of the lung wet-to-dry weight ratio and MPO activity, and a significant improvement in arterial oxygen partial pressure (PaO₂).

A(1–7) also protects against oleic acid (OA)-induced ALI/ARDS in Sprague-Dawley rats. In this study, animals in Group 1 (control) did not receive any pharmacological interventions. In Group 2, ALI was induced by intravenous infusion of 0.2 mg/kg OA in the absence of any treatment. In Group 3 (OA+A(1–7)), ALI was induced as in Group 2, and infusion of A(1–7) at 5 pmol/kg per minute was
initiated 30 min after ALI induction. A(1–7) attenuated the development of OA-induced ARDS, as demonstrated by the fact that A(1–7) infusion abrogated OA-induced changes in lung wet-to-dry weight ratio and MPO activity, and significantly reduced increases in BAL protein concentration and pulmonary vascular resistance (PVR).

In a more recent study, A(1–7) was able to improve pulmonary function, including prolonged improvement in oxygenation, reduction in inflammatory cells recruitment and reduction in lung fibrosis long term in an animal model of ARDS involving two insults, acid instillation and prolonged injurious ventilation. Notably, Ang-(1–7) was effective even after delayed administration.17

**Benefits of A(1–7) in diabetes, pneumonia and systemic organ failure**

Diabetic patients are at higher risk for infection and severe complications from SARS-CoV-2 infection.18 Increased oxidative stress and chronic inflammation has deleterious effects on kidney, heart and lung health in these patients; this is only more exacerbated with SARS-CoV-2 infection.19,20 Long and short-term administration of A(1–7) treatment in diabetic mice has improved lung, heart and kidney function by reducing oxidative stress and inflammation.21–24 In diabetic mice, Resident alveolar macrophages are depleted in diabetic mice and restored to normal levels with A(1–7) treatment.24 Further, data from SARS patients suggests that disease severity is correlated with an immune dysregulation in neutrophil clearance commonly seen in diabetic patients. Again, A(1–7) has been shown to restore proper pathogen clearance and immune resolution of neutrophils. Results from an unpublished study using a bacterial model of pneumonia, show that treatment with A(1–7) reduced lung congestion 24 h after infection (Figure 2).

Finally, in an unpublished study of bone marrow transplant following lethal irradiation, mice began to die due to an unplanned norovirus infection at day 3 after transplant. In mice that received donor cells from the saline treated animals and saline treatment after transplant, there was only 20% survival (Figure 3). In A(1–7) treated mice (both donor and recipient), there was 100% survival. These data show that A(1–7) not only ameliorates sequelae secondary to infection, but also prevents multiple organ system failure and death.

**Conclusion**

There is an immediate need for treatments to help patients fighting this COVID-19 pandemic. Beyond this pandemic, there is need for therapeutics for future pandemics that are not pathogen specific and act by supporting natural healing processes. A(1–7) can act by several mechanisms to improve overall outcomes in respiratory infections; specifically anti-fibrotic properties and immune resolution that are very important in lung health. Further, A(1–7) has also shown to be effective in ameliorating systemic organ damage caused by oxidative stress and inflammation, both important contributors of death in severe infections like this COVID-19 pandemic.

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