HYPOTHESIS LETTER: Protease-activated receptor 1 (PAR1): A target for repurposing in the treatment of COVID-19?

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Abstract

In the search to rapidly identify effective therapies that will mitigate the morbidity and mortality of COVID-19, attention has been directed towards the repurposing of existing drugs. Candidates for repurposing include drugs that target COVID-19 pathobiology, including agents that alter angiotensin signaling. Recent data indicate that key findings in COVID-19 patients include thrombosis and endothelitis Activation of PAR1 (protease activated receptor 1), in particular by the protease thrombin, is a critical element in platelet aggregation and coagulation. PAR1 activation also impacts on the actions of other cell types involved in COVID-19 pathobiology, including endothelial cells, fibroblasts and pulmonary alveolar epithelial cells. Vorapaxar is an approved inhibitor of PAR1, used for treatment of patients with myocardial infarction or peripheral arterial disease. Here, we discuss evidence implying a possible beneficial role for vorapaxar in the treatment of COVID-19 patients and in addition, other as-yet non-approved antagonists of PAR1 and PAR4.

Abbreviations

ACE2: Angiotensin Converting Enzyme 2; ANG II: Angiotensin II; ALI: Acute Lung Injury; ARDS: Acute Respiratory Distress Syndrome; AGTR1: Angiotensin II Receptor 1; EMT: Epithelial-to-Mesenchymal transition; TF: Tissue Factor; PAR1 and PAR4: protease activated receptor 1 and protease activated receptor 4

Main Text

Recent studies have provided evidence that the pathobiology of COVID-19 includes thrombosis and endothelitis (Ackermann et al., 2020; Joly et al., 2020; Magro et al., 2020; *hematology.org*). Such evidence includes data indicating infection by SARS-CoV-2 of ACE2-expressing endothelial cells in infected tissue (Ackermann et al., 2020), widespread endothelial inflammation and disruption (Ackermann et al., 2020; Joly et al., 2020), and alterations in the coagulation cascade that can result in thrombosis in COVID-19 patients. This thrombosis is associated with increased circulating levels of fibrin and D-dimer (indicative of increased coagulation cascade activity), in parallel with increased levels of inflammatory markers (Joly et al., 2020; Magro et al., 2020; *hematology.org*). Reports that indicate an association of COVID-19 with risk of stroke, even in younger patients (Oxley et al., 2020), are consistent with the idea that the pathophysiology of COVID-19 involves damage to the endothelium and associated thrombosis. Thrombosis can be a feature of pulmonary infections, acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) (Frantzeskaki et al., 2017). Hence, the administration of anti-coagulants in ALI and ARDS (besides in COVID-19) is under study (Camprubí-Rimblas et al., 2018).

Protease-activated receptor 1(PAR1), a G protein-coupled receptor (GPCR), is a key mediator of the aggregation and activation of platelets and as such, an initiator of the coagulation cascade. Thrombin, the major physiologic agonist of PAR1, cleaves its amino terminal, thereby exposing a tethered ligand that self-activates

the receptor; other proteases can also activate PAR1 (Heuberger & Schuepbach, 2019). PAR1 is widely expressed, including in cell types relevant to COVID-19 pathobiology, including pneumocytes, endothelial cells, fibroblasts and platelets. PAR4, another PAR receptor, is also expressed on human platelets and other cell types and when activated, also activates platelets (Heuberger & Schuepbach, 2019).

Vorapaxar is a selective PAR1 antagonist that is approved for the treatment of patients with myocardial infarction and/or peripheral arterial disease. The action of vorapaxar in inhibiting platelet aggregation is thought to be the main therapeutic action of this drug, with limited data on effects of vorapaxar on other cell types (Heuberger & Schuepbach, 2019). Atopaxar, another PAR1 antagonist, has a shorter half-life than vorapaxar and has been tested in phase II trials. No approved drugs currently target PAR4 but several PAR4 inhibitors have been identified (*guidetopharmacology.org* , Armstrong et al., 2020).

Based on the expression and physiological effects of PAR1 (and potentially, PAR4) in cell types relevant to COVID-19 pathobiology, the following questions arise:

1. Does PAR1 (and perhaps PAR4) contribute to the pathophysiology of COVID-19?
2. Might antagonism of PAR1 (or perhaps PAR4) reduce pathological effects of COVID-19, especially ones associated with thrombosis and related features of the infection?
3. Do patients being treated with vorapaxar and exposed to SARS-CoV-2 have an altered susceptibility to developing COVID-19, its clinical features and course?

Thrombin is produced *in-vivo* from pro-thrombin, as part of the coagulation cascade. A key component of the extrinsic mechanism of this cascade is the production of tissue factor (TF) (**Figure**). Hyperinflammation associated with severe COVID-19 disease can promote TF production by endothelial cells, macrophages and fibroblasts (Joly et al., 2020). Hence, this disease setting is likely associated with elevated TF and thrombin production and PAR1 activation, as in patients with ALI/ARDS, as noted above.

Besides its action in platelets, PAR1 regulates endothelial function. The emerging paradigm is a dose-dependent effect of thrombin on endothelial cells: at low concentrations, thrombin (via PAR1) is protective, whilst at high concentrations, thrombin promotes endothelial dysfunction and disruption (Bae et al., 2009; Jose et al., 2014; Jose & Manuel, 2020). Data for effects of PAR1 in alveolar and bronchial epithelial cells in the lung suggest that PAR1 activation drives a pathological phenotype, including epithelial-to-mesenchymal transition (EMT), apoptosis and secretion of inflammatory factors (e.g., Asokananthan et al., 2002; Suzuki et al., 2005; Song et al., 2013; Atanelishvili et al., 2014). PAR1 also promotes lung fibrosis, including by stimulating pro-fibrotic processes in fibroblasts, increasing their transformation to myofibroblasts and secretion of extracellular matrix proteins (e.g., Blanc-Brude et al, 2005; Atanelishvili et al., 2014; Jose et al., 2014). As discussed previously (Sriram & Insel, 2020), dysregulation of the angiotensin pathway , in particular, elevated ANG II signaling is likely a key mediator of COVID-19 pathobiology. The effects of PAR1 on fibroblasts, endothelial cells and epithelial cells are similar to those of ANG II via AGTR1 (**Figure** , adapted from Sriram & Insel, 2020), raising the possibility that such effects may be additive or synergistic.

By contrast, sparse direct evidence exists for effects of PAR1 on immune cells. The lack of immune-associated adverse effects with use of vorapaxar (Morrow et al., 2012) suggests that an impact on immune cells is unlikely a major component of effects of thrombin-PAR1 signaling in COVID-19. As recently noted (Jose & Manuel, 2020), studies with mice suggest that inhibition of PAR1 may also reduce inflammation and enhance host immune response, including with viral infection, although the cell types involved in these responses are unclear.

The pathobiology of COVID-19 thus suggests that PAR1 may be a therapeutic target in COVID-19. Importantly, one could repurpose vorapaxar or expand trials with atopaxar. However, concerns regarding the safety of PAR1 antagonists require pre-clinical validation of a role of PAR1 in COVID-19 models prior to clinical trials. Increased bleeding risk, which can include fatal bleeding events, is the major adverse effect of vorapaxar (Morrow et al., 2012). An advantage of atopaxar is fewer bleeding events and its shorter half-life compared to that of vorapaxar, which has such a slow rate of metabolism (Statkevich et al., 2010; Heuberger & Schuepbach, 2019) that its effects, including potential adverse effects, are essentially irreversible within the

time frame (˜7 days) relevant to treatment of the acute, imminently life-threatening effects of COVID-19.

Given these hazards, caution is necessary in evaluating the potential of PAR1 inhibition as a means to treat COVID-19 patients. Preclinical studies need to evaluate effects of PAR1 antagonists (and similarly, for tool compounds for inhibition of PAR4) on alveolar epithelial cells, endothelial cells and fibroblasts along with assessment of these drugs*in-vivo* in animal models of COVID-19. It is as-yet unclear if clinical features of COVID-19, associated with thrombosis are replicated in animal models. Nevertheless, the growing recognition of endothelitis and thrombosis in COVID-19 patients provides a strong incentive to determine the potential utility of PAR1 (and perhaps PAR4) inhibitors to improve the outcome of such patients. Besides investigating such approaches, it would be of interest to assess methods to directly deliver PAR receptor antagonists to the lungs, via inhalation-based methods, as a possible way to mitigate systemic adverse effects.

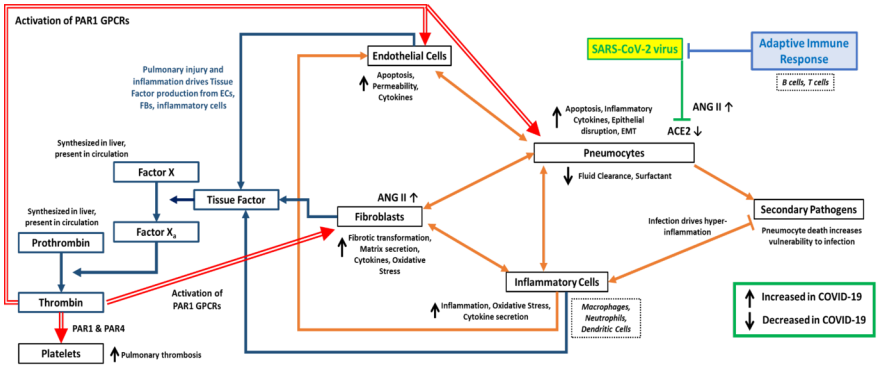

Figure : COVID-19 pulmonary pathobiology is driven by dysregulation of angiotensin signaling (adapted from Sriram & Insel, 2020), which results in feedback between various cell types, leading to increased inflammation and cell death. These conditions are associated with increased Factor X activation, resulting in formation of thrombin, which has actions on platelets, endothelial cells, fibroblasts and alveolar epithelial cells inducing similar effects to those of ANG II in several cell types and promoting thrombosis, which exacerbates pulmonary injury along with that of other organs. ACE2: Angiotensin converting enzyme 2; ANG II: Angiotensin II; EMT: Epithelial-to-Mesenchymal transition.

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