

## HYPOTHESIS

# Proteinase-activated receptor 1: A target for repurposing in the treatment of COVID-19?

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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 signalling. Recent data indicate that key findings in COVID-19 patients include thrombosis and endotheliitis. Activation of proteinase-activated receptor 1 (PAR1), in particular by the serine 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. We discuss evidence for a possible beneficial role for vorapaxar in the treatment of COVID-19 patients and other as-yet non-approved antagonists of PAR1 and proteinase-activated receptor 4 (PAR4).

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## 1 | HYPOTHESIS

Recent studies have provided evidence that the pathobiology of COVID-19 includes thrombosis and endotheliitis (Ackermann et al., 2020; American Society of Hematology, 2020; Joly, Siguret, & Veyradier, 2020; Magro et al., 2020). 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 fibrinogen/fibrin degradation products and D-dimer (indicative of increased coagulation cascade activity), in parallel with increased levels of inflammatory markers (American Society of Hematology, 2020; Joly et al., 2020; Magro et al., 2020). 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 and acute respiratory distress syndrome (Frantzeskaki, Armaganidis, & Orfanos, 2017). Hence, the administration of anticoagulants in acute lung injury and acute respiratory distress syndrome (besides in COVID-19) is under study (Camprubi-Rimblas, Tantinyà, Bringué, Guillaumat-Prats, & Artigas, 2018).

**Proteinase-activated receptor 1 (PAR1)**, a GPCR, is a key mediator of the aggregation and activation of platelets and, as such, an initiator of the coagulation cascade. **Thrombin**, a serine protease and the major physiological 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. Proteinase-activated receptor 4 (PAR4), another PAR receptor, is also expressed on human platelets and other cell types; agonist stimulation of PAR4 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 inhibition of platelets by vorapaxar 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; Alexander et al., 2019; Bunnett et al., 2019).

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

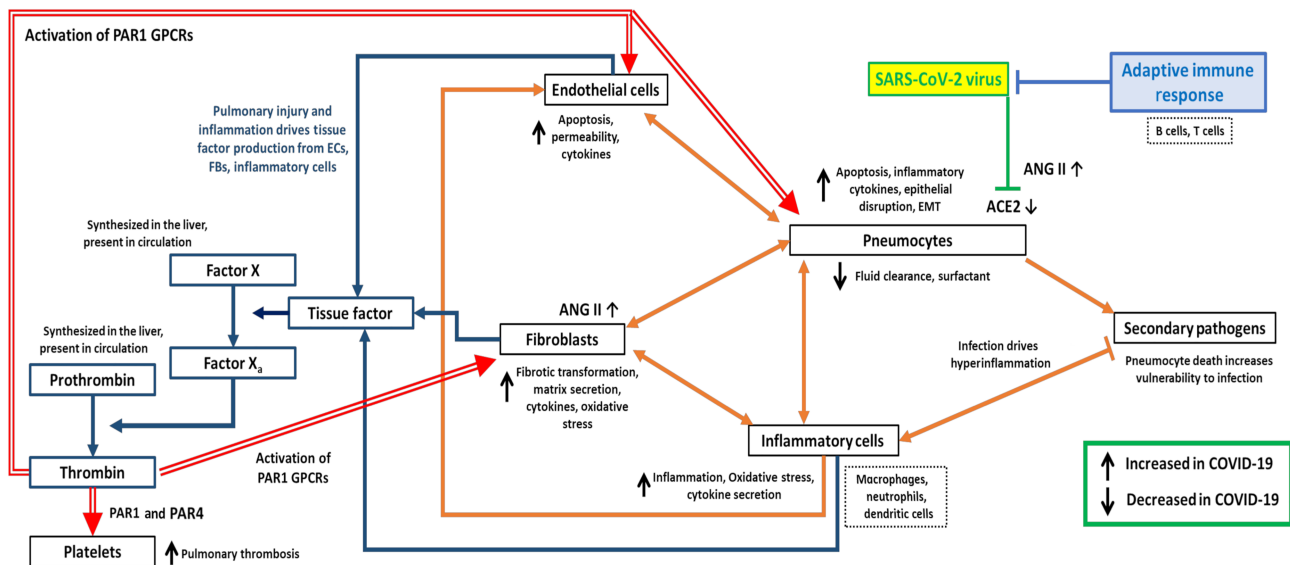
- Does PAR1 (and perhaps PAR4) contribute to the pathophysiology of COVID-19?
- Might antagonism of PAR1 (or perhaps PAR4) reduce pathological effects of COVID-19, especially ones associated with thrombosis and related features of the infection?
- Do patients being treated with vorapaxar and exposed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

Thrombin is produced *in vivo* from prothrombin, as part of the coagulation cascade. A key component of the extrinsic mechanism of this cascade is the production of tissue factor (Figure 1).

Hyperinflammation associated with severe COVID-19 disease can promote tissue factor production by endothelial cells, macrophages and fibroblasts (Joly et al., 2020). Hence, this disease setting is likely associated with elevated tissue factor and thrombin production and PAR1 activation, as in patients with acute lung injury and acute respiratory distress syndrome, 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, Kim, Park, & Rezaie, 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. Asokanathan et al., 2002; Atanelishvili et al., 2014; Song, Kang, Park, & Yoon, 2013; Suzuki et al., 2005). 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. Atanelishvili et al., 2014; Blanc-Brude et al., 2005; José, Williams, & Chambers, 2014). As discussed previously (Sriram & Insel, 2020), dysregulation of the angiotensin pathway, in particular, elevated angiotensin II (ANG II) signalling, 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 angiotensin II via AT<sub>1</sub> receptor (Figure 1, 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



**FIGURE 1** COVID-19 pulmonary pathobiology is driven by dysregulation of angiotensin signalling (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 (ECs), fibroblasts (FBs) and alveolar epithelial cells inducing similar effects to those of angiotensin II (ANG II) in several cell types and promoting thrombosis, which exacerbates pulmonary injury along with that of other organs. EMT, epithelial-to-mesenchymal transition; PAR1, proteinase-activated receptor 1; PAR4, proteinase-activated receptor 4

of vorapaxar (Morrow et al., 2012) suggests that an impact on immune cells is unlikely a major component of effects of thrombin–PAR1 signalling 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 in viral infection, although the cell types involved in these responses are unclear. Debate exists regarding the role of PAR1 in infectious disease models (Posma et al., 2019). Early reports with mouse models with PAR1 knockout and/or overexpression gave contradictory results. Antoniak et al. (2013) suggested that PAR1 has a protective role in viral infection, whereas Khoufache et al. (2012) obtained the opposite findings. These different results may be explained by differences in viral load that animals were exposed to in these studies (Posma et al., 2019). Numerous subsequent studies support the idea that PAR1 has a pathological role in inflammatory and infectious disease (e.g. Aerts et al., 2013; Lê et al., 2018; Yang & Tang, 2016, among others; reviewed in Posma et al., 2019). The plurality of data from *in vivo* studies thus implies that PAR1 inhibition has a potentially beneficial effect in infections, including viral respiratory infections.

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 preclinical 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 with that of vorapaxar, which has such a slow rate of metabolism (Heuberger & Schuepbach, 2019; Statkevich et al., 2012) 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. Recent work by Motta et al. (2019) demonstrated a protective role for thrombin in maintaining segregation between intestinal epithelium and gut microbiota (an effect that may occur in other epithelia, including in the lung), implying that inhibition of thrombin may have deleterious effects related to the microbiome. Such potential effects require further study in preclinical models.

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 animal models of COVID-19. It is as yet unclear if clinical features of COVID-19, associated with thrombosis, are replicated in animal models. The development of animal models for COVID-19 is an ongoing effort, including work with ferrets, rhesus monkeys and other organisms (Sriram & Insel, 2020). We anticipate greater clarity on the suitability of specific animal models for mimicking the clinical features of COVID-19, as data on these animal models accumulate. Preclinical studies with animal models should help to further define the mechanisms for potential beneficial effects of PAR1 inhibition *in vivo*, for example, actions on platelets or other cell types. Such data

are available in mouse models of acute lung injury and acute respiratory distress syndrome (e.g. reviewed by Frantzeskaki et al., 2017) and can be extended to COVID-19, in relevant animal models as these become better established. *In vivo* studies should optimally be complemented by *ex vivo* studies with isolated cells from treated animals, in order to confirm target engagement and functional effects in specific cellular populations.

Nevertheless, the growing recognition of endotheliitis 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.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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