Therapeutic Potential of Targeting Plasminogen Activator Inhibitor-1 in COVID-19

Tahsin F. Kellici, Ewa S. Pilka, Michael J. Bodkin

PII: S0165-6147(21)00053-5

DOI: https://doi.org/10.1016/j.tips.2021.03.006

Reference: TIPS 1808

To appear in: *Trends in Pharmacological Sciences*

Please cite this article as: T.F. Kellici, E.S. Pilka and M.J. Bodkin, Therapeutic Potential of Targeting Plasminogen Activator Inhibitor-1 in COVID-19, *Trends in Pharmacological Sciences* (2021), https://doi.org/10.1016/j.tips.2021.03.006

Therapeutic Potential of Targeting Plasminogen Activator Inhibitor-1 in COVID-19

Tahsin F. Kellici[1]*, Ewa S. Pilka[1], Michael J. Bodkin[1]

Evotec (UK) Ltd, Oxfordshire, United Kingdom, Tel. +44(0)1235441207

*Corresponding author: tahsin.kellici@evotec.com

ABSTRACT

Latest research show that SERPINE1 overexpression plays an important role in COVID-19-associated coagulopathy leading to acute respiratory distress syndrome (ARDS). Ways to target this protein remain elusive. In this forum, we discuss recent evidence linking SERPINE1 with COVID-19 related ARDS and summarize the available data on inhibitors of this target.

SERPINE1 Structure and Function

Plasminogen Activator Inhibitor-1 (PAI-1 aka SERPINE)1 is a typical member of the Serpin family of proteins with a molecular weight of 45 kDa, 9 α-helices and 3 β-sheets [1]. SERPINE1 acts as a "suicide inhibitor" under physiological conditions, binding both tissue type and urokinase plasminogen activator (tPA and uPA) and is regulated by human neutrophil elastase [2-4]. As such, SERPINE1 prevents the formation of plasmin, and inhibits fibrinolysis and blood clot dissolution. SERPINE1 is synthesized in the active form (with the reactive center loop (RCL) in the position showed in Figure **1A**). This form has a half-life of 1-2 hours in the physiological environment, but it can increase several fold when bound to Vitronectin (VN). This modulation has attracted attention due to the important role that it plays in two vital pathways: a pro-tumorigenic role in cancer due to the pro-angiogenic and anti-apoptotic effects [3], as well as a crucial role in keeping the balance between rates of plasminogen activation and fibrin degradation [4]. It is in this second role as a primary regulator of fibrinolysis in plasma that SERPINE1 is implicated in the symptoms of COVID-19 (Figure **1B**).

SERPINE1 Overexpression in COVID-19

The first evidence that high levels of SERPINE1 in circulatory system are associated with coronavirus infection started emerging during the Severe Acute Respiratory Syndrome (SARS) epidemic in 2003. A brief article by Wu *et al.*, highlighted that the plasma levels of SERPINE1 in SARS patients were elevated (355 ng/ml) when compared to patients with infectious pneumonias of other causes (88 ng/ml) or healthy controls (61 ng/ml). VN levels in blood plasma were also extremely elevated when compared to healthy controls (1,538 mg/l vs 310 mg/l) [5]. As a consequence, SERPINE1 is in its active state for longer.

A similar picture is painted for the case of the SARS-Cov2 (COVID-19) pandemic. In a recent single-center study, exploratory analyses of haemostatic factors were performed on 68 COVID-19patients. There were near-universal elevations in SERPINE1 levels among critically and non-

critically ill patients. This is another indication that normal fibrinolysis is prevented in COVID-19 associated coagulopathy [6]. Another study of the fibrinolytic parameters of 78 COVID-19 patients showed that the observed hypofibrinolysis was immediately related with increased SERPINE1 presence. The level of the protein in intensive care unit (ICU) patients was 96 ng/ml while for the non-ICU patients, the concentration was 77 ng/ml. Both values are elevated when compared to its normal range (4-43 ng/mL). Similar increase was observed for tPA (24 ng/ml vs 2-12 ng/ml [normal range]). Presence of fibrin deposition in the lung parenchyma of COVID-19 patients suggests that even though the amount of tPA is elevated, high SERPINE1 levels can overcome local tPA release [7]. This observation is further strengthened by a recent article by Cugno *et al.*, where they have found increased tPA and PAI-1 levels in all patients suffering from COVID-19 regardless of disease severity [8]. The evidence suggests that lethal SARS-Cov2 infection hijacks the normal profibrinolytic signaling and leads to overall dysfunction in the system, including increased SERPINE1 expression and severe lung disease [9]. This is further validated by a study that reports that high levels of tPA (in particular) and PAI-1 were associated with mortality and a significant enhancement in spontaneous *ex vivo* clot-lysis [10]. While all this evidence makes a compelling case for the importance of SERPINE1 in the symptoms of COVID-19, it must be noted that the available publications and data on elevated SERPINE1 levels in COVID-19 do not distinguish between active and latent forms.

Inhibition of SERPINE1

Nebulized fibrinolytic agents such as monoclonal antibodies or small molecule inhibitors targeting SERPINE1 have been found to considerably enhance the bronchoalveolar fibrinolytic system as well as easing the symptoms of ARDS by elevating the levels of plasmin that effectively removes fibrin [11]. Many inhibitors of SERPINE1 have been developed in the past, both antibodies/nanobodies and small molecule inhibitors. Nanobodies have been used for both stabilizing SERPINE1 and modulate its activity. Recently VHH-s-a93 was shown to be a potent inhibitor of SERPINE1 with an IC_{50} of 7 nM [12]. As the nanobody binds to the reactive center loop, it also acts independently from the binding of VN to the serpin. MEDI-579 is another antibody that binds to the RCL independently of VN and was found to treat normal human lung fibroblasts with a $pIC_{50} = 9.8 \pm 0.14$ (10.5 nM) [13]. Small molecule inhibitors like Tiplaxtinin and Aleplasinin were the first orally efficacious inhibitors of SERPINE1 developed by Wyeth. They were used extensively in clinical trials on subjects with Alzheimer's disease. The activity of

both these drugs depends on whether SERPINE1 is bound to VN, suggesting overlapping binding sites. As VN levels are highly elevated during ARDS, this kind of inhibitor is unlikely to treat hypofibrinolysis in the lungs efficiently. On the other hand, small molecules such as MDI-2268, CDE-096 and TM5614 are Vitronectin independent inhibitors (Figure **1A**) [14, 15]. Although pre-clinical and clinical trials (for indications other than ARDS) are ongoing for these compounds and showing promising activity, none of them are yet in advance stage, leaving plenty of room for improvement. Common side-effect such as localized uncontrolled bleeding confirms increased fibrinolysis upon SERPINE1 inhibition. The important aspect would be combining feasibility of direct administration to the respiratory tract with good permeability to the bloodstream.

One further indication that regulated SERPINE1 production is beneficiary to patients suffering from COVID-19 is shown by Kang *et al*. [16]. In their study, seven severe COVID-19 patients exhibited decreased serum SERPINE1 levels and improved clinical features (including lower C-reactive protein levels) after treatment with Tocilizumab, an arthritis drug repurposed for SARS-Cov2 [17].

While a procoagulant shift has been observed when the symptoms of COVID-19 become more serious in later stages, it must be noted that plasmin can also promote viral infection by cleaving proteins that play an important part in cell infection, mainly in the earlier stages of the disease. In a recent study by Metcalf *et al*., it has been suggested that the need for enhancement of fibrinolytic activity mainly occurs in stage 3 of the course of COVID-19 [18]. That would mean that the use of SERPINE1 modulators could have a beneficial effect in the later and more serious symptoms of the coronavirus infection. This point though remains still controversial as another observational study emphasizes that earlier administration of tPA to COVID-19 patients yields better results [19].

Conclusions

One of the consequences of SARS-Cov2 interfering with ACE2 is hypofibrinolysis. Even the smallest blood clots within lungs can cause a lasting damage and it is important to get this COVID-19 symptom under control. In this forum article, we have summarized the available data indicating SERPINE1 as a promising target for treating severe cases of COVID-19. We have

also reviewed the range of SERPINE1 inhibitors available to date and stressed, despite promising results, the need for further development for some of the existing therapeutics.

Conflict of interest

All the authors are employees of Evotec (UK) Ltd.

References

1. Kellici, T.F. et al. (2020) Small-molecule modulators of serine protease inhibitor proteins (serpins). Drug Discov Today, 10.1016/j.drudis.2020.11.012.
2. Wu, K. et al. (1995) The cleavage and inactivation of plasminogen activator inhibitor type 1 by neutrophil elastase: the evaluation of its physiologic relevance in fibrinolysis. Blood 86 (3), 1056-1061, 10.1182/blood.V86.3.1056.1056.
3. Kubala, M.H. and DeClerck, Y.A. (2019) The plasminogen activator inhibitor-1 paradox in cancer: a mechanistic understanding. Cancer Metastasis Rev. 38 (3), 483-492, DOI: 10.1007/s10555-019-09806-4.
4. Urano, T. et al. (2019) Recognition of Plasminogen Activator Inhibitor Type 1 as the Primary Regulator of Fibrinolysis. Curr. Drug Targets 20 (16), 1695-1701, DOI: 10.2174/1389450120666190715102510.
5. Wu, Y.P. et al. (2006) Analysis of thrombotic factors in severe acute respiratory syndrome (SARS) patients. Thromb. Haemost. 96 (1), 100-1, DOI: 10.1160/TH05-12-0827.
6. Goshua, G. et al. (2020) Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study. Lancet Haematol. 7 (8), e575-e582, DOI: 10.1016/s2352-3026(20)30216-7.
7. Nougier, C. et al. (2020) Hypofibrinolytic state and high thrombin generation may play a major role in SARS-COV2 associated thrombosis. J. Thromb. Haemost., DOI: 10.1111/jth.15016.
8. Cugno, M. et al. (2021) Complement activation and endothelial perturbation parallel COVID-19 severity and activity. J Autoimmun 116, 102560, 10.1016/j.jaut.2020.102560.
9. D'Alonzo, D. et al. (2020) COVID-19 and pneumonia: a role for the uPA/uPAR system. Drug Discov. Today, DOI: 10.1016/j.drudis.2020.06.013.
10. Zuo, Y. et al. (2021) Plasma tissue plasminogen activator and plasminogen activator inhibitor-1 in hospitalized COVID-19 patients. Sci. Rep. 11 (1), 1580, 10.1038/s41598-020-80010-z.
11. Hofstra, J.J. et al. (2013) Nebulized fibrinolytic agents improve pulmonary fibrinolysis but not inflammation in rat models of direct and indirect acute lung injury. PLoS One 8 (2), e55262, DOI: 10.1371/journal.pone.0055262.
12. Sillen, M. et al. (2020) Structural Insights into the Mechanism of a Nanobody That Stabilizes PAI-1 and Modulates Its Activity. Int. J. Mol. Sci. 21 (16), DOI: 10.3390/ijms21165859.
13. Vousden, K.A. et al. (2019) Discovery and characterisation of an antibody that selectively modulates the inhibitory activity of plasminogen activator inhibitor-1. Sci. Rep. 9 (1), 1605, DOI: 10.1038/s41598-019-38842-x.
14. Rouch, A. et al. (2015) Small molecules inhibitors of plasminogen activator inhibitor-1 - an overview. European Journal of Medicinal Chemistry 92, 619-36, 10.1016/j.ejmech.2015.01.010.

15. Reinke, A.A. et al. (2019) Dual-reporter high-throughput screen for small-molecule in vivo inhibitors of plasminogen activator inhibitor type-1 yields a clinical lead candidate. J Biol Chem 294 (5), 1464-1477, 10.1074/jbc.RA118.004885.
16. Kang, S. et al. (2020) IL-6 trans-signaling induces plasminogen activator inhibitor-1 from vascular endothelial cells in cytokine release syndrome. Proc Natl Acad Sci U S A 117 (36), 22351-22356, 10.1073/pnas.2010229117.
17. Guaraldi, G. et al. (2020) Tocilizumab in patients with severe COVID-19: a retrospective cohort study. The Lancet Rheumatology 2 (8), e474-e484, 10.1016/s2665-9913(20)30173-9.
18. Medcalf, R.L. et al. (2020) Fibrinolysis and COVID-19: A plasmin paradox. J. Thromb. Haemost., DOI: 10.1111/jth.14960.
19. Orfanos, S. et al. (2020) Observational study of the use of recombinant tissue-type plasminogen activator in COVID-19 shows a decrease in physiological dead space. ERJ Open Res 6 (4), 10.1183/23120541.00455-2020.

Figure Legends:

Figure 1: A) **SERPINE1 structure and its inhibitors.** Tiplaxtinin and Aleplasinin are VN-dependent, CDE-096, TM5614 and MDI-2268 are not. However, precise binding pockets for these inhibitors have not been identified. B) **Fibrinolysis in COVID-19**. Angiotensin-converting enzyme 2 (ACE2) converts angiotensin II (AngII) to angiotensin 1–7 (Ang1-7) under normal circumstances. When ACE-2 is hindered by SARS-Cov2, this pathway is affected, increasing the levels of Ang2 in the blood. AngII and the angiotensin II receptor 1 (AT1) enhance the release of SERPINE1 (PAI-1) by endothelial cells. Due to the high concentration of VN, most of SERPINE1 is probably bound to it. SERPINE1 inhibits tPA and as a result the conversion of plasminogen to plasmin is limited, leading to hypofibrinolysis.