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REVIEW ARTICLE

A lesson from a saboteur: High-MW kininogen impact in coronavirus-induced disease 2019

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The newly identified coronavirus SARS-CoV-2 that spread from China is causing the pandemic COVID-19 with a fatality rate from 5-15%. It causes fever, cough, myalgia, fatigue up to dyspnoea, responsible for hospitalization and artificial oxygenation. SARS-CoV-2 infects human cells using ACE2, the transmembrane protease serine 2 (TMPRSS2) and the SARS-CoV-2 main protease (M^{pro}). Once bound to ACE2 and the other two proteases in concert they allow the virus replication and spread throughout the body. Our attention has been focused on the role of ACE2 as its binding to by the virus increases bradykinin and its metabolites, which facilitate inflammation in the lung (causing cough and fever), coagulation and the complement system. These three systems are involved in angioedema, cardiovascular dysfunction and sepsis, pathologies which occur in COVID-19 patients. Thus, we propose that blocking the kallikrein–kinin system with lanadelumab, approved for hereditary angioedema, will prevent facilitation of these 3 systems.

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1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a newly identified coronavirus that emerged for the first time in the city of Wuhan and rapidly spread through China to cause a disease known as coronavirus disease 2019 (COVID-19) (http://www.who.int/csr/don/12-january-2020-novel-coronavirus-china/en/). Because the outbreak of COVID-19 has rapidly spread worldwide, affecting millions of people, the World Health Organization (WHO) has declared SARS-CoV-2 as a global pandemic (https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19-11-march-2020).

SARS-CoV-2 is a new *Betacoronavirus* belonging to the same subgroup as severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV), which caused the severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) outbreaks in 2002 and 2012, respectively (Chen, Liu, & Guo, 2020). Several studies have identified a sequence homology of 79.5% between SARS-CoV-2 and SARS-CoV (Wu et al., 2020; Zhou et al., 2020). Therefore, SARS-CoV-2 genome

Abbreviations: [des-Arg^9]BK, [des-Arg^9]bradykinin; 2′-*O*-MTase, 2′-*O*-ribose methyltransferase; ALT, alanine aminotransferase; ARDS, acute respiratory distress syndrome; B_1 receptor, bradykinin receptor 1; BK, bradykinin; CFR, case fatality rate; COVID-19, coronavirus disease 2019; DMARDs, disease-modifying antirheumatic drugs; eNOS, endothelial NOS; HIV, human immunodeficiency virus; HMWK, high-MW kininogen; hrsACE2, human recombinant soluble ACE2; ICU, intensive care unit; iNOS, inducible NOS; INSTIs, integrase strand transfer inhibitors; KKS, kallikrein–kinin system; LMWK, low-MW kininogen; Lys-BK, lysyl-bradykinin; mAbs, monoclonal antibodies; MERS, Middle East respiratory syndrome; MERS-CoV, Middle East respiratory syndrome coronavirus; M^{pro}, main protease; PAI-1, plasminogen activator inhibitor 1; PD, peptidase domain; PGI2, PG I2; RAS, renin–angiotensin system; RBD, receptor-binding domain; S, spike; SARS, severe acute respiratory syndrome; SARS-CoV, severe acute respiratory syndrome coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TF, tissue factor; TMPRSS2, transmembrane protease serine 2; uPA, urokinase-type plaminogen activator; WHO, World Health Organization.

sequencing was rapidly performed, leading to the rapid availability of real-time PCR diagnostic test, which is actually used to identify infected subjects allowing for epidemiological tracking (Corman et al., 2020). SARS-CoV-2 is a single-stranded RNA virus characterized by an envelope-anchored **spike glycoprotein** (S), which drives virus entry into target cells by binding to specific membrane proteins of sensitive cells leading to viral replication (Xu et al., 2020).

Epidemiological data indicate that SARS-CoV-2 infection progresses through human-to-human contact, which is predominantly realized via droplet transmission (Ong et al., 2020). As reported by WHO, the incubation period for SARS-CoV-2 is 2–14 days, although a longer period may be at the basis of asymptomatic and subclinical infection (https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf), whereas illness establishment mainly occurs in 10 days (Guan et al., 2020). Although the estimated case fatality rate (CFR) of COVID-19 floats from 5% to 15%, the number of deaths is very high.

Several reports have summarized the clinical and epidemiological features of patients affected by COVID-19. In the first published cohort of 41 laboratory-confirmed cases infected with SARS-CoV-2 (Huang et al., 2020), it was reported that infected patients had a median age of 49.0 years and 73% of them were men. The common symptoms are fever (98%), cough (76%), myalgia, and/or fatigue (44%). Dyspnoea occurs within 8 days from the establishment of these symptoms in 55% of these patients. Very few COVID-19 patients have gastrointestinal symptoms. The most prominent symptoms being upper respiratory tract ones, indicating that the target cells might be located in the upper and lower airways. All hospitalized patients show abnormalities in chest CT images, which are characterized by grinding glass-like and consolidation areas, in 98% of the cases reporting bilateral lungs impairment as the basis of bilateral interstitial pneumonia. Because of respiratory complications, around 32% of COVID-19 patients are admitted to intensive care unit (ICU). The morbidity is mainly due to respiratory failure typical of acute respiratory distress syndrome (ARDS), but the mortality is due to underlying multiple organ failure due to alteration in coagulation with ensuing thrombosis and embolism, with the consequences of septic shock and/or cardiovascular alterations (Huang et al., 2020).

2 | BIOLOGICAL TARGETS FOR SARS-CoV-2

One key discovery in understanding the secrets of SARS-CoV-2 infection involves the viral spike protein, which binds to the host **ACE2** via the recognition of the receptor-binding domain (RBD) (Sriram & Insel, 2020; Zhou, Yang, et al., 2020), a similar mechanism that is used by SARS-CoV to mediate infection (Sriram & Insel, 2020; Zhou, Yang, et al., 2020). The viral attachment to ACE2 is the first of a multistep process, the next one is mediated by cleavage by cellular proteases of the spike protein at the S1/S2 and S2 site (Chen, Guo, Pan, & Zhao, 2020; Letko, Marzi, & Munster, 2020). As in the case of SARS-CoV (Li, Li, Farzan, & Harrison, 2005), the virus receptor-binding domain comprised of a S1 subunit, which directly interacts with the peptidase domain (PD) of ACE2 causing a tighter and higher binding of the virus to the host cell. So far, three mutations (V367F, W436R and D364Y) of the receptor-binding domain on SARS-CoV-2 have been correlated to higher human ACE2 affinity, ensuing higher infectivity (Ou et al., 2020). Therefore, the localization of ACE2 is very relevant to identify of the viral route to the particular host cells (Sriram & Insel, 2020). Besides type II pneumocytes (Zhao et al., 2020), other organs, that is, heart, oesophagus, kidney, bladder, ileum, oral cavity and testes express ACE2, explaining why some COVID-19 patients also exhibit non-respiratory symptoms. To date, in the attempt to find a potential drug against COVID-19, human recombinant soluble ACE2 (hrsACE2) was proposed to prevent viral attachment (Monteil et al., 2020; Sriram & Insel, 2020). However, phase 1 and 2 clinical trials results demonstrated a lack of therapeutic effect on COVID-19, most likely due to its biological nature or because ACE2 is just the tip of the iceberg.

Another key event for virus entrance into the host is represented by the cellular **transmembrane protease serine 2** (TMPRSS2) that drives the spoke protein priming (Hoffmann et al., 2020). TMPRSS2 is a cell surface protein from the serine protease transmembrane family type II that is broadly expressed on epithelial cells (Xu et al., 2020; Zou et al., 2020) and is involved in the cleavage of the SARS-CoV and influenza virus haemagglutinin protein (Böttcher et al., 2006). Hoffmann et al. (2020) found that SARS-CoV-2 uses both TMPRSS2 and endosomal cysteine proteases **cathepsin B** and **L** (CatB/L) to enter host cells. The inhibition of TMPRSS2 by means of **camostat** mesilate, an TMPRSS2 inhibitor, partially blocked SARS-CoV-2 entry while, camostat mesilate and E-64d also known as **aloxistatin** , an inhibitor of CatB/L, completely prevented virus endocytosis *in vitro* (Alexander et al., 2020; Hoffmann et al., 2020).

Other lines of research are focusing their attention on the **SARS-CoV-2 main protease** (M^{pro}), a cysteine protease present in the coronavirus replicase polyprotein (Zhou et al., 2019). This protease plays a critical role both in the immune regulation and in viral replication, in that it regulates the proteolytic cleavage of polyproteins. M^{pro} drives the cleavage of polyproteins pp1a and pp1ab, which in turn are responsible for the generation of functional proteins such as RNA polymerase, endoribonuclease and exoribonuclease (Khan et al., 2020). For this reason, it has been speculated that M^{pro} could represent an attractive target for COVID-19 treatment. In this context, two different molecular docking and molecular dynamic simulation studies revealed four drugs that could act against M^{pro}:- the antibacterial drug talampicillin, the antipsychotic drug **lurasidone** (Elmezayen, Al-Obaidi, Şahin, & Yelekçi, 2020) and the antiviral drug raltegravir and paritaprevir, which were already used in the antiretroviral therapy against the human immunodeficiency virus (HIV) infections, as integrase strand transfer inhibitors (INSTIs) (Khan et al., 2020). M^{pro} also cleaves the 2′-*O*-ribose methyltransferase (2′-*O*-MTase), a protein that catalyses the methylation of 5′-terminal cap structure of viral mRNAs (Chen et al., 2011). Because this reaction is crucial for viral replication and expression in host cells (Menachery et al., 2014), 2′-*O*-MTase was suggested as another possible druggable target for COVID-19 treatment (Khan et al., 2020), although it is still unclear whether 2′-*O*-MTase, as well as M^{pro}, contributes to SARS-CoV-2 infection.

3 | ACE2 AND BRADYKININ

ACE2 is a membrane-associated aminopeptidase and belongs to the ACE family of dipeptidyl carboxydipeptidases and has high homology to human **ACE** (Tipnis et al., 2000). Secreted ACE2 cleaves **angiotensin I** into **angiotensin-(1–9)** and **angiotensin II** into the vasodilator **angiotensin-(1–7)** (Patel, Zhong, Grant, & Oudit, 2016; Sriram & Insel, 2020). Beyond its role in the cardiovascular system, it plays a role in the regulation of renal function and fertility (Koitka, Cooper, Thomas, & Tikellis, 2008; Pan, Zhan, Le, Zheng, & Jin, 2013). Once SARS-CoV-2 binds to ACE2, the enzyme is blocked, therefore, leading to what we are actually observing in terms of high blood pressure in COVID-19 patients and pulmonary oedema up to angioedema, which underlies the fact that physiologically ACE2 also cleaves several other bioactive peptides, among which is **[des-Arg^9]bradykinin** ([des-Arg^9]BK) (Donoghue et al., 2003; Vickers et al., 2002) (Figure 1). Herein, besides the interference with the renin–angiotensin system (RAS) (Imai et al., 2005; Kuba et al., 2005; Sriram & Insel, 2020), increasing inflammation and vascular permeability also occur due to the increased activity of [des-Arg^9]bradykinin that binds to **bradykinin 1 receptor** (B_1 receptor), which can lead to acute lung inflammation (Sodhi et al., 2018; Sriram & Insel, 2020) (Figure 1). The activation of the [des-Arg^9]bradykinin/B_1 receptor axis induces the release of pro-inflammatory chemokines (i.e. **CXCL5**, **CCL2** and **CXCL1**) and cytokines (i.e. **TNF-α**, **IL-1β** and **IL-6**), exacerbating lung inflammation/oedema up to organ dysfunction (Sodhi et al., 2018). Therefore, as already suggested by van de Veerdonk et al. (2020), the cytokine storm observed in COVID-19 may underlie an impaired breakdown of [des-Arg^9]bradykinin, paving the way for the pharmacological blockade of B_1 receptor signalling has a treatment.

Instead, in this review, we want to focus our reader's attention on the upstream signalling that leads to the production of **bradykinin**. The kallikrein–kinin system (KKS) consists of a complex interaction between prekallikrein and high-MW kininogen (HMWK) (Hooley, McEwan, & Emsley, 2007) (Figure 2). High-MW kininogen is a multifunctional single-chain plasma glycoprotein, primarily expressed by the liver and secreted into the bloodstream. High-MW kininogen consists of six different protein domains (Shariat-Madar & Schmaier, 1999) and binds to prekallikrein by means of a sequence in domain 6. Then the detachment of the domain 4 releases bradykinin (Griffin & Cochrane, 1979). Kallikreins are serine proteases responsible for the release of kinins which are vasoactive peptides that cause vascular smooth muscle relaxation and an increase in vascular permeability (Bhoola, Figueroa, & Worthy, 1992). It has been found that kallikrein exists in two different forms, **kallikrein B_1**, also known as plasma kallikrein, which cleaves high-MW kininogen into bradykinin and in turn interacts with the constitutive **B_2 receptor** and tissue kallikrein, which processes low-MW kininogen (LMWK) into **Lys-bradykinin** known as kallidin. The interaction of bradykinin or kallidin with B_1 and B_2 receptors will increase the activation of both

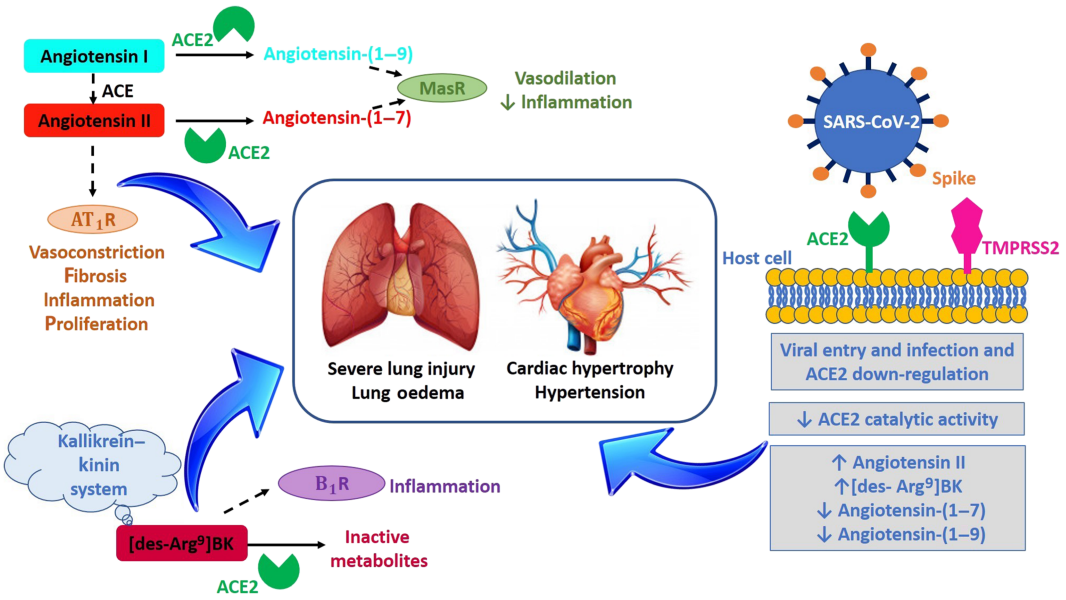

FIGURE 1 ACE2 function and its regulation in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. ACE2 is a carboxypeptidase that catalyses and inactivates angiotensin I and angiotensin II, respectively, into the vasodilator peptides angiotensin-(1–9) and angiotensin-(1–7), which bind **Mas receptor** (MasR) leading to reduced inflammation and vasodilation. ACE2 also cleaves [des-Arg^9]bradykinin ([des-Arg^9]BK), a bioactive kinin derived from kininogen pathway, into inactive metabolites. ACE2 is the cell entry target for SARS-CoV-2. The binding of viral spike glycoprotein with ACE2 and the priming of the spike through the transmembrane protease serine 2 (TMPRSS2) lead to SARS-CoV-2 infection. The binding of SARS-CoV-2 down-regulates ACE2 expression, leading to a reduction of its enzymatic activity and the ensuing increase of angiotensin II and [des-Arg^9]BK levels. Angiotensin II takes its deleterious effect by binding the angiotensin II type 1 receptor (AT_1R), whereas [des-Arg^9]BK concurs to inflammation by binding bradykinin 1 receptor (B_1R), resulting in severe lung injury, pulmonary inflammation and oedema, increased coagulation, hypertension and cardiac hypertrophy, which are all features of coronavirus disease 2019 patients

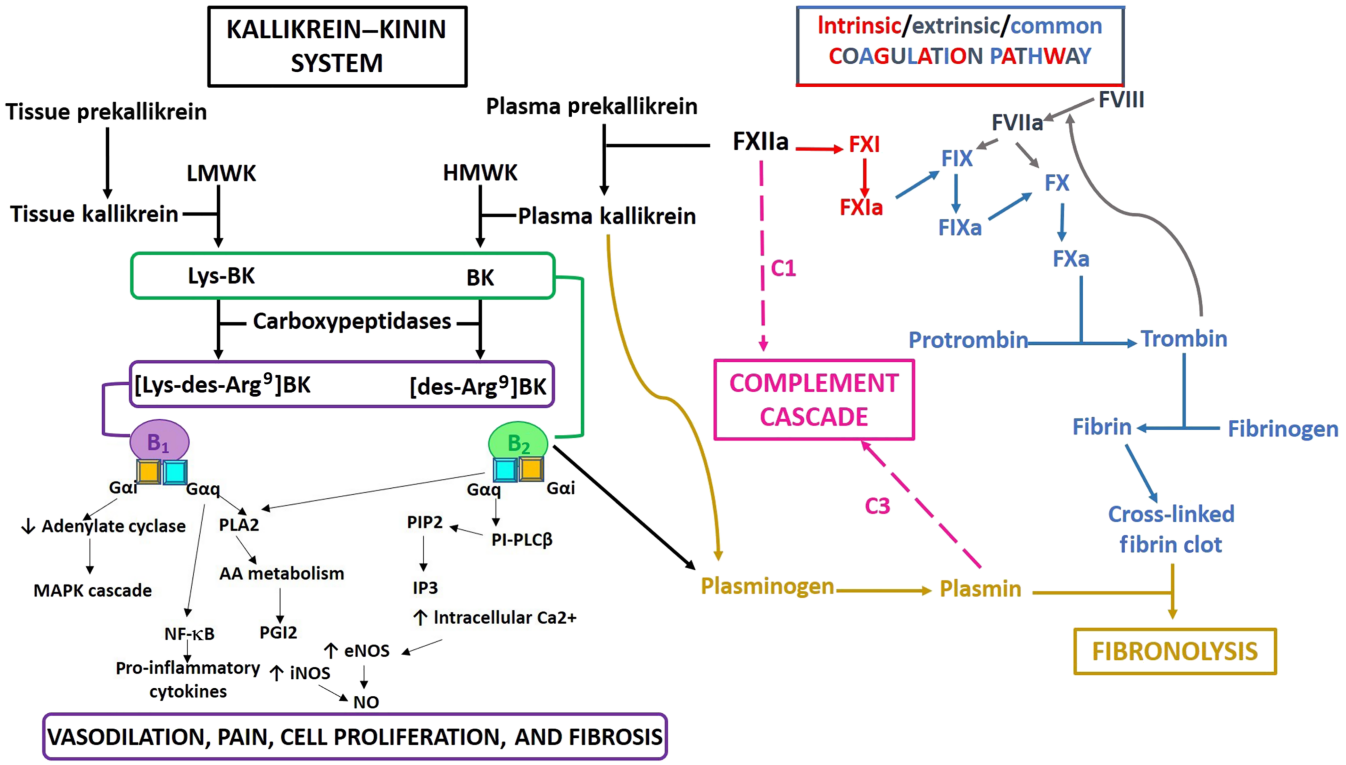

FIGURE 2 Crosstalk between kallikrein–kinin system (KKS), coagulation, fibrinolysis, and complement cascade. KKS (black box and arrows) consists of tissue and plasma kallikrein, which act on high-MW kininogen (HMWK) and low-MW kininogen (LMWK) to generate bradykinin (BK) and kallidin (Lys-BK). BK and Lys-BK, and their metabolites [des-Arg^{10}]kallidin ([Lys-des-Arg^{9}]BK) and [des-Arg^{9}]BK), act via two G-coupled receptors, B_1 and B_2 receptors, resulting in increased vascular permeability, vasodilation, oedema formation and ultimately hypotension. Plasma kallikrein, which is induced by the reciprocal activation of the factor XIIa (FXIIa) and plasma prekallikrein, also influences the fibrinolytic pathway by activating plasminogen into plasmin and leading to fibrin degradation and D-dimer generation (yellow box and arrows). Beyond its role in KKS, FXIIa starts the intrinsic coagulation pathway (red arrows). Blood coagulation consists of an intrinsic and extrinsic (grey arrows) pathways, both resulting in activation of the coagulation factor X (FX), which subsequently leads to thrombin and fibrin generation (common pathway; blue arrows). The coagulation cascade is also a starting point for the complement system (pink box and arrows). FXIIa binds C1q component of the complement triggering the classic pathway; moreover, plasmin activation, which is also promoted via B_2 receptor signalling, triggers C3 cleavage inducing the activation of both lecithin and extrinsic pathways of the complement

endothelial NOS (eNOS) and **inducible NOS** (iNOS), with an ensuing release of **NO**, a potent vasodilator, and of **prostacyclin** (PGI_2) along with pro-inflammatory cytokines/chemokines responsible for acute inflammation, causing vasodilation, pain, cell proliferation and fibrosis (Kuhr, Lowry, Zhang, Brovkovych, & Skidgel, 2010; Tsai, Hao, Chen, Lin, & Wu, 2015), symptoms typical of COVID-19 (Figures 1 and 2).

Plasma and tissue kallikrein are initially secreted as inactive, but both of them are activated by serine protease activity (Bhoola et al., 1992). The reciprocal activation of **coagulation factor XII**a (Hageman factor) and plasma prekallikrein promotes the activation of kallikrein, which, besides the catabolism of high-MW kininogen into bradykinin, initiates the intrinsic pathway of coagulation, influencing fibrinolysis (Figure 2). At the same time, tissue prekallikrein cleaves low-MW kininogen into **[des-Arg^{10}]kallidin** ([Lys-des-Arg^{9}]bradykinin) and [des-Arg^{9}]bradykinin, which interact with B_1 receptors further enhancing inflammation. The intrinsic pathway of coagulation is then correlated to the extrinsic pathway in that factor XIIa activates **coagulation factor XI**, which in turn activates **coagulation factor IX**, which subsequently leads into the common pathway by means of **coagulation factor X** and then **thrombin**, with the generation of fibrin aggregates, hence the need to detect D-dimer, a fibrin degradation product, in COVID-19 patients (Figure 2). In this context, studies in rat models that express both bradykinin receptors show, *in vitro*, that bradykinin acting through the B_2 receptor on the surface of endothelial cells promotes the expression of procoagulant and antifibrinolytic proteins, such as **platelet activating** factor (tissue factor) and plasminogen activator inhibitor 1 (PAI-1) (Kimura et al., 2002). On the other hand, plasma kallikrein can align **urokinase-type plasminogen activator** (uPA) in such close proximity as to drive **plasminogen** activation into plasmin, which degrades fibrin aggregates (Selvarajan, Lund, Takeuchi, Craik, & Werb, 2001), effects that are widely observed in sepsis, another co-morbidity of COVID-19. However, it has been shown that the complex high-MW kininogen and factor XIIa can also bind to one of the three endothelial cell-binding sites, the 33-kDa cell surface receptor for the first component of complement C1q (gC1qR/p33), which has high affinity for high-MW kininogen (Ghebrehiwet, CebadaMora, Tantral, Jesty, & Peerschke, 2006). Therefore, the activation of the classical complement pathway together with the activation of plasmin causing the

conversion of C3 into C3a and C3b, which induces the activation of both lecithin and extrinsic pathways of the complement system with the ensuing activation of humoral immunity, exacerbating the inflammatory process (Figure 2).

These events may happen in COVID-19 patients from the early onset up to the severe level of the pathology. To date, the above pathological conditions are typical of angioedema, cardiovascular dysfunction and sepsis, which symptoms occur in COVID-19 patients. But it is obvious to ask the correlation between these symptoms and the viral infection. Why would this happen? As above reported (van de Veerdonk et al., 2020), the viral blockade of ACE2 inhibits not only the degradation of angiotensin II but also the degradation of bradykinin. Therefore, because bradykinin is derived from high-MW kininogen and because kallikrein–kinin system leads to the coagulation and complement activation, we believe that the alteration of plasmatic kallikrein could serve as potential pharmacological target.

4 | FURTHER THERAPEUTIC HYPOTHESES FOR COVID-19 PATIENTS

In the attempt to identify the effective anti-SARS-CoV-2 therapy, many therapeutic approaches have been proposed. In particular, ongoing clinical trials are focusing on two big branches, the antiviral drugs, which aim to diminish viral replication, and the disease-modifying antirheumatic drugs (DMARDs) and immunotherapeutic agents to hijack the cytokine storm that the virus is able to induce. Encouraging clinical trials indicate that remdesivir (Grein et al., 2020) and neutralizing monoclonal antibodies (mAbs: i.e. tocilizumab and sarilumab) (Xu et al., 2020; http://www.news.sanofi.us/2020-03-16-Sanofi-and-Regeneron-begin-global-Kevzara-R-sarilumab-clinical-trial-program-in-patients-with-severe-COVID-19) are a promise for fighting COVID-19.

It has to be pointed out that all the ongoing clinical trials include monitoring of coagulation parameters such as D-dimer, which is a metabolite of fibrin aggregates. Although there are no published case series reporting abnormal coagulation parameters in hospitalized severe COVID-19 patients, in a multicentre retrospective cohort study in China, elevated D-dimer levels (>1 g/L) were strongly associated with in-hospital deaths from severe COVID-19 (Zhou et al., 2020). To date, low-MW heparin (LMWH), enoxaparin, has been proposed for these patients either to avoid thromboembolism events (Tang et al., 2020) or to inhibit the cytokine storm (Shi et al., 2020), due to non-anticoagulant fraction of enoxaparin suppresses *in vitro* IL-6 and IL-8 (CXCL8) release from human pulmonary epithelial cells (Shastri et al., 2015). Moreover, both *in vitro* and *in vivo* experimental studies have shown that human coronaviruses utilize heparin sulfate proteoglycans for attachment to target cells (Milewska et al., 2014). Indeed, interaction between the SARS-CoV-2 spike S1 protein receptor-binding domain (SARS-CoV-2 S1 RBD) and heparin has been recently showed, suggesting a role for heparin in the therapeutic armamentarium against COVID-19 (Mycroft-West et al., 2020).

So far, the published clinical observations of biochemical markers in COVID-19 patients include elevated LDH, D-dimer, bilirubin, high levels of pro-inflammatory cytokines that accompany interstitial pneumonia and renal and cardiac injury due to thromboembolic events, which also underlie septic shock that occurs in severe COVID-19 patients. Therefore, based on what is described above and cross-linking biochemical with clinical outcomes, in this review, we propose another therapeutic approach based on the inhibition of the kallikrein–kinin system.

Lanadelumab is a monoclonal antibody against the plasmatic kallikrein, which is important for the cleavage of high-MW kininogen into bradykinin and is involved in the coagulation as well as in the induction of the complement system (Figure 2). Actually, lanadelumab is used for the treatment of angioedema and there have been no reports of adverse and severe events, other than hypersensitivity, myalgia, and hepatic alteration of alanine aminotransferase (ALT) (https://www.ema.europa.eu/en/documents/assessment-report/takhzyro-epar-public-assessment-report_en.pdf). The rationale to suggest lanadelumab is in that this mAb can block the upstream axis that leads to kinin formation (van de Veerdonk et al., 2020), avoiding the inflammatory and coagulation storm besides the complement system in SARS-CoV-2-infected patients, likely preventing the exacerbation of COVID-19, in parallel with antiviral therapy.

Lanadelumab has never been used to control COVID-19 symptoms. However very recently, an open controlled trial entitled "Lanadelumab for treatment of COVID-19 disease" was registered, in order to generate the proof of concept (https://www.clinicaltrialsregister.eu/ctr-search/trial/2020-002472-12/NL#summary). In particular, one of the goals of this trial is to demonstrate the safety of the dose of 300 mg, injected intravenously. Most likely, the choice of this dose is based on the positive results in that to prevent acute angioedema attacks in patients with type I and type II hereditary angioedema (HAE) (https://clinicaltrials.gov/ct2/show/record/NCT02586805).

In conclusion, we believe that the blockade of ACE2 increases not only the activity of angiotensin II on the cardiovascular system but also the levels of [des-Arg^9]bradykinin derived by high-MW kininogen. Therefore, the hypothesis to block the production of [des-Arg^9]bradykinin upstream by blocking the metabolism of high-MW kininogen could be another option to face this tremendous pandemic event that affects lifestyle in the whole world, obliging to social limitations and stay-at-home politics.

4.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander et al., 2019).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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