1874-3404/21

The Open Toxicology Journal

Content list available at: https://opentoxicologyjournal.com

CLINICAL TRIAL STUDY

Blood Hemostasis Dysfunction and Inflammation in COVID-19 Patients: Viral and Host Active Molecules as Therapeutic Targets

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Abstract:

The COVID-19 pandemic is challenging world health authorities and researchers. WHO is supervising many clinical studies to ascertain whether some known drugs can be effective against the disease. Meanwhile, researchers around the globe are working on cellular and molecular mechanisms that are key steps of SARS-Cov-2 associated infection. Blood hemostasis dysfunction, inflammation, hypoxia and venous thrombotic events are reported to be involved in the pathophysiology of COVID-19 patients at early and late severe stages of the disease. It is of high relevance to understand how SARS-Cov-2 triggers negative cellular and biochemical events in infected persons. A large number of cell species and active molecules, such as blood and tissue enzymes, cytokines, and other active amines and lipid inflammatory molecular species, can be involved in immune reactions and host defense mechanisms upon human infectious diseases or other kinds of health issues such as trauma or snake envenomation. Possible physiopathology trends of COVID-19 and some therapeutic perspectives are discussed in the present minireview.

Keywords: SARS-Cov-2, COVID-19, Hemostasis, Blood, Coagulation, Fibrinolysis, Inflammation, Serine proteases.

Article History	Received: May 07, 2020	Revised: September 18, 2020	Accepted: November 11, 2020

1. INTRODUCTION

The COVID-19 pandemic caused by SARS-Cov-2 took place early this year after a first patient was diagnosed with ARSD in Wuhan (China). The virus molecular characterization was reported and its target receptor was identified [1, 2] as the membrane angiotensin-converting enzyme-2. Researches on effective drug therapy were launched in clinical studies like the Solidarity Trial and the Discovery Trial in Europe. Both clinical trial studies include many classic antiviral and antiretroviral molecules, anti-malaria drugs, cytokines, antibodies and Chinese plant extracts [3 - 5]. Recent clinical findings have found that COVID-19 patients can suffer from hypoxia [6], pulmonary venous thromboembolism [7], and gut dysbiosis [8 - 10]. Similar viral infection-associated dysbiosis was described earlier upon viral infections [11]. SARS-Cov-2 - associated respiratory distress syndrome is the leading cause of death in COVID-19 patients. However, the underlying mechanisms that cause death are still not totally known.

Recent scientific reports have shown that the severity of COVID-19 symptoms, as well as the mortality caused by COVID-19, would significantly affect the population after 40 years of age, and this incidence increases with age [12]. The highest death figures (virus fatality index) are among those over 60 years of age, reaching 15.6% in those over 80 years of age [13]. On the other hand, individuals with underlying health conditions are more vulnerable than those without. Obese patients and those who have health issues such as cardiovascular diseases, diabetes, chronic respiratory diseases, hypertension or cancer have a higher case fatality rate for COVID-19 [14].

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2. PHYSIOPATHOLOGY ASSOCIATED WITH SARS-COV-2 INFECTION

2.1. Inflammation

Blood analyses of patients have shown “cytokines storm”, which might be leading to a serious general inflammation [15, 16]. A very large number of pro-inflammatory cytokines are described in viral infections. Pro-inflammatory cytokines are released by specialized immune cells (Fig. **1**), and this phenomenon might exacerbate a patient's health weakness. It is likely that in COVID-19 patients, many blood cell species are activated, such as platelets, monocytes and neutrophils. Other tissue resident cells such as alveolar macrophages, mesangial cells or glial cells might be activated as well. A large number of active lipid [17] inflammatory mediators (prostaglandins, leukotrienes, platelet activating factor) and edema associated molecules (histamine, serotonin and bradykinin) can play a role in the inflammatory process of COVID-19. Macrophages and

neutrophils are the main cell species that release NADPH-Oxidase dependent oxygen free radicals upon their activation. Hence, oxidative stress might be an additional burden in the physiopathology of SARS-Cov-2 infection [18]. Interestingly, recent studies, including a meta-analysis investigation [19], have shown that intravenous corticoid (anti-inflammatory steroids) treatment was associated with a better outcome of COVID-19 severe cases.

2.2. Hemostasis Dysfunction

Clinical and laboratory data of COVID-19 patients from many countries have pointed out several molecular and cellular mechanisms that may be crucial in the physiopathology caused by SARS-Cov-2 [20, 21]. It was observed that patients of COVID-19 have hypoxia and hemoglobin oxygen transport dysfunction. On the other hand, venous thromboembolism might be damaging tissues in several organs, such as kidneys, heart, lungs and brain. The hallmark of SARS-Cov-2 infection and thrombogenic blood parameters was associated with the severity of COVID-19. Blood coagulation dysfunction [22, 23] and thrombocytopenia [24] were reported in COVID-19 patients. Blood clots were found in many organs, and abnormally elevated levels of plasmin [25] and D-dimers [23] were found in severe cases of COVID-19 patients, and tissue factor was

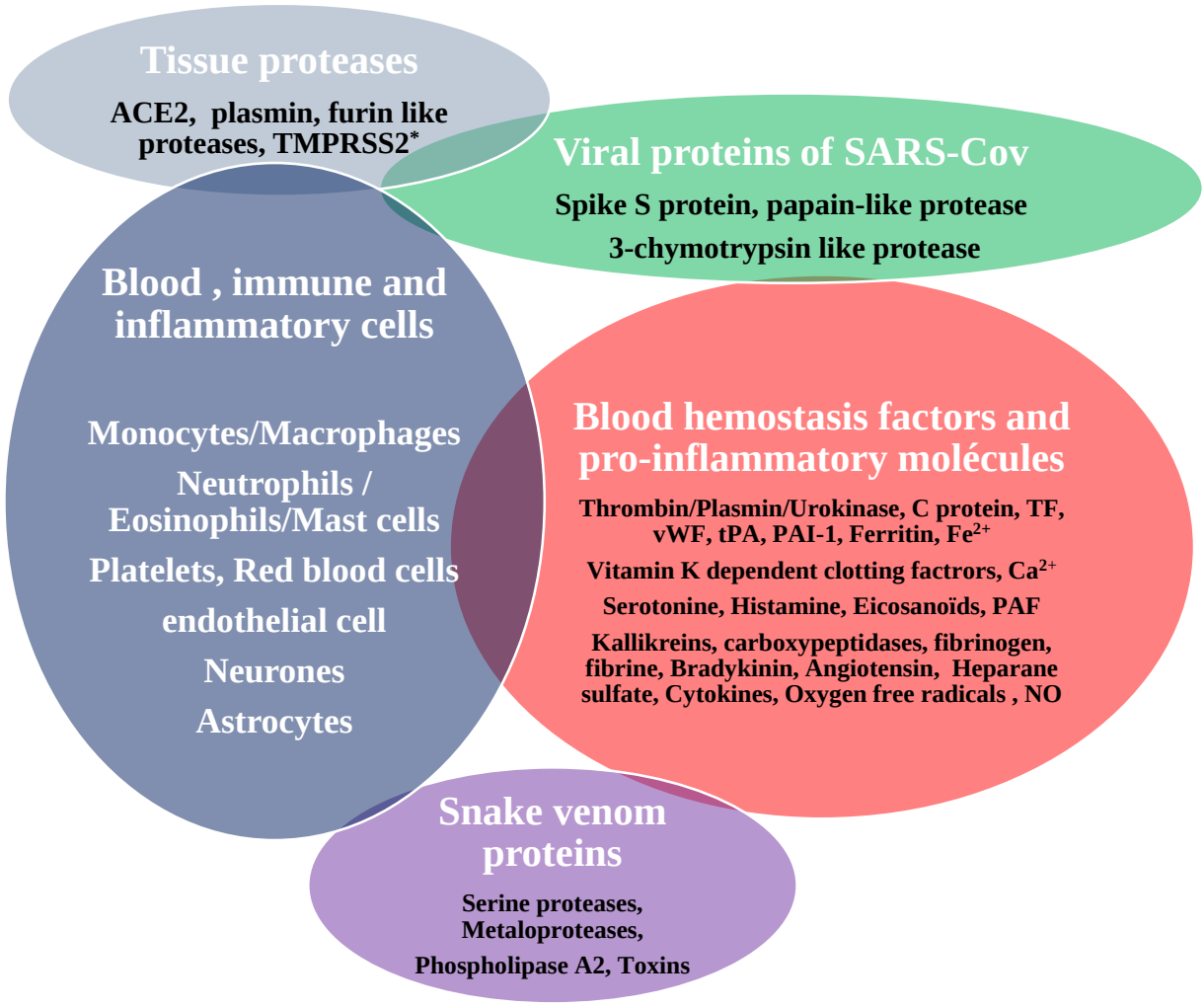

Fig. (1). The figure represents groups of cells and molecules (Blood and tissue host cells, viral enzymes, snake venom enzymes) that could have close enzymatic and pharmacological interactions. The red ellipse represents blood components, clotting/fibrinolysis factors, and inflammatory mediators that are the resulting output of cells and enzymatic activations in the host body under physiological conditions or during pathological situations. Viral proteins and enzymes have close interaction with the host cells, starting from the priming of the S spike protein by host serine proteases. Blood coagulation/fibrinolysis enzymatic system involves many protease activities. Snake venom proteases, phospholipases and toxins interact with many host cells and blood components that lead to hemostasis dysfunction. The ellipse forms do not imply separate physical compartments in the host body. It is meant that foreign enzymes and molecules of invading organisms can pharmacologically interact with host enzymatic and molecular machinery, which leads to physiopathological events. * TMPRSS2: Transmembrane protease serine2. ACE2: Angiotensin converting enzyme 2. TF: Tissue factor. vWF: von Willebrand factor. tPA: Tissue plasminogen activator. PAI-1: Plasminogen activator inhibitor. PAF: platelet activating factor. NO: Nitric oxide.

described as a possible key molecule in coagulation dysfunction in COVID-19 patients [26, 27]. Human blood coagulation and fibrinolysis are controlled by many serine protease enzymes (Fig. **1**). The physiological process of blood clotting and fibrinolysis is highly regulated by a large number of blood proteins that have enzymatic proteolytic activities, such as thrombin and plasmin, to mention just these two cornerstone enzymes. Blood hemostasis is a specific target in many infectious diseases and health issues such as envenomation. Indeed, it is known that blood clotting induced by snake venom is associated with many serine protease enzymes of the venom [28]. The toxicity of snake venom is known to be associated with a large number of venom enzymes, such as phospholipases, proteases and other toxins. Among SARS-Cov-2 proteins, papain-like protease and 3-chy -motrypsin-like protease are key proteases for their replication and infectivity [29 - 31]. Blood coagulation and fibrinolysis in COVID-19 patients could be affected by the above viral proteases in the case of their release in the host bloodstream. They would act through similar human blood clotting/fibrinolysis protease cascade (thrombin, plasmin) or that of snake venoms protease-induced hemostasis dysfunc-tions [28].

2.3. Serine Protease-dependent Entry of the Virus in Host Cells

Entry of the SARS-Cov-2 in host cells was shown to involve the priming of the S spike protein of the virus by a host cell serine protease (Fig. **1**). Thus, surface proteases of the host cell play a key role in the infectivity of viruses (Fig. **2**). In the case of SARS-Cov-2, TMPRSS2 (Transmembrane protease serine2), a human serine protease, primes the S virus protein [32], and leads to the subsequent binding of the virus to its receptor; Angiotensin Converting Enzyme 2. Other host proteases could play a role in priming the S spike protein as well [33]. Research on the molecular sequence of SARS-Cov-2 RNA and its spike glycoprotein sequence have shed light on other host cell serine protease enzymes that could participate in priming the S protein on host cells [34, 35]. The above studies have shown that SARS-Cov-2 RNA, unlike its coronavirus predecessors, has a genomic sequence of 12 bases, which encodes a peptide sequence of a few amino acids that represents a cleavage site for several serine proteases found in the entire human organism. The sequence of the SARS-Cov-2 S glycoprotein would explain why the virus can infect most of the organs, and this would explain the actual higher virulence of SARS-Cov-2 and its widespread effects on the bloodstream, lungs and other organs. Interestingly, plasmin was also described as a possible priming enzyme of the S viral protein during SARS-Cov-2 infection [25], and its possible inhibition was suggested to be one of the therapeutic targets against SARS-Cov-2.

2.4. Perspectives in Therapeutic Targeting of SARS-Cov-2 - Associated Pathology

Due to recent clinical and biological findings on COVID-19 cited above, the physiopathology and clinical aspects of COVID-19 were much better understood, which allowed better therapeutic management of the disease, especially in severe cases. In the absence of a specific antiviral drug against SARS-Cov-2, anti-bacterials, blood thinners and corticoids were the main drugs that gave some hope, mainly in COVID-19 severe cases.

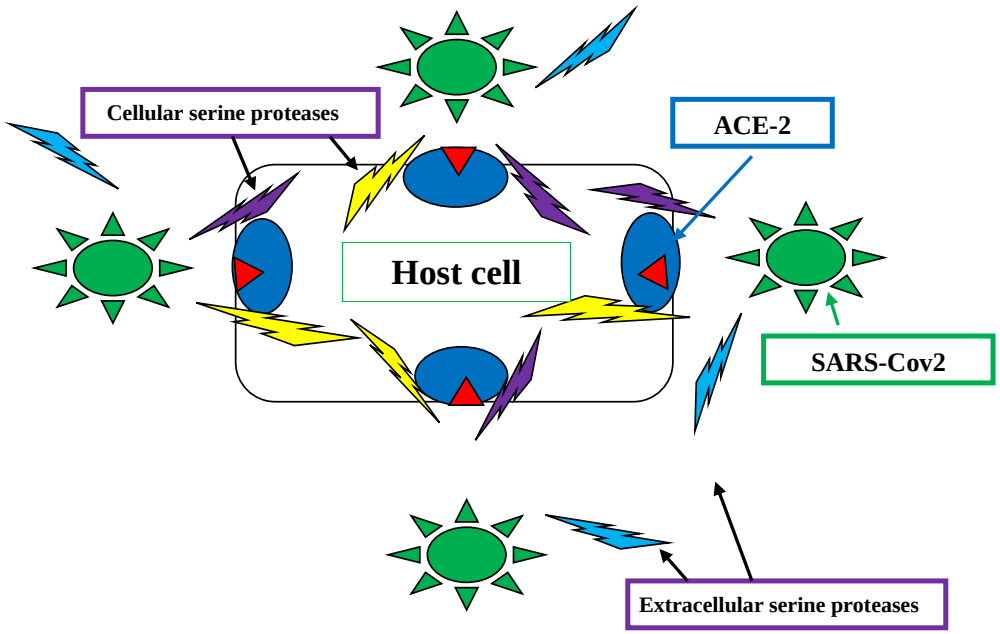

Fig. (2). A host cell bearing receptors of SARS-Cov-2; ACE-2. Cell infection by the virus involves priming of the S spike glycoprotein of the virus by cellular serine proteases and possibly by other extracellular serine proteases. Priming of the S spike protein allows the virus to pursue its entry into the host cell, and proceed to the rest of the biochemical steps for its RNA and protein synthesis, followed by virion assembly.

For virus targeting, and according to the above mentioned possible physiopathological processes that could be associated with SARS-Cov-2 infection (Fig. **1**), as most of the molecular mechanisms of the viral infection involve catalytic activities of proteases, starting from the first beginning of viral S Spike protein priming, until virus replication and assembly, it should be obvious to target several protease activities on both host cells and viral enzymatic machinery. Viral protease inhibition was used as a therapeutic strategy for many viral infections, such as the HIV ones [36]. Recently, molecular modeling studies have suggested some old protease inhibitors for COVID-19 therapy [37]. Therapeutic strategies that use nucleotide and nucleoside drugs that interfere with viral replication are presented elsewhere [38], and will not be discussed in this paper.

Promising therapeutic strategies for COVID-19 are being suggested, such as lactoferrin milk enzyme [39 - 43] and oligosaccharides [44 - 48] as next antivirals in human infectious diseases, as these substances have exhibited antiviral activities in laboratory experiments. Interestingly, in a recent clinical study on COVID-19 patients in Italy [43], lactoferrin has been used to treat mild-to-moderate and asymptomatic COVID-19 patients to prevent disease evolution. The study concluded that lactoferrin induced an early viral clearance and a fast clinical symptoms recovery, in addition to a statistically significant reduction of D-Dimer, Interleukin-6 and ferritin blood levels. Hence, lactoferrin might be a real promising therapeutic molecule either as a purified active ingredient or as part of a whole natural product.

In relationship to traditional medicine, some natural food and plant extracts, known for their antiviral, anti-oxidant, anti-inflammatory and anti-cancer properties, were recently proposed as therapeutic candidates for the management of COVID-19. These potential therapeutic candidates include camel milk, a known traditional food of many countries in Asia and Africa, where people use it for both nutrition and healing purposes. The health benefits and therapeutic properties of camel milk are well documented in many reviews [49 - 51]. Among camel milk active components, lactoferrin was extensively studied for its anti-viral and antibacterial properties [39, 52, 53], and gut bacteria and immune modulatory properties [54]. Lactoferrin was shown to have a serine protease activity that could have some biochemical relevance in its anti-viral properties [40]. Lactoferrin was also described to possess anti plasminogen activity, which could play a role in the control of blood clot and fibrinolysis [41].

Milk oligosaccharides have anti-viral properties [45 - 48, 55, 56], in part, due to their carbohydrate binding on viral glycoproteins. Oligosaccharides and lactoferrin are both present in camel milk, and the latter was suggested as a functional diet for COVID-19 management [57]. However, the effect of whole camel milk ingestion intended for antiviral and antibacterial effects could involve other molecules than oligosaccharides and lactoferrin.

Lectins of plant and seaweed origins are known for their interaction with carbohydrate moieties of glycoproteins and for interacting with viruses [58]. Many lectins were previously shown to bind the S glycoprotein of coronaviruses [59] and gave promising results in laboratory experiments against viral infection [60]. Interestingly, in recent laboratory experiments, a lectin from edible hyacinth beans was shown to block the infections of Influenza and SARS-Cov-2 *in vitro* and *in vivo* [61]. Many laboratory experiments [62, 63] have shown binding and antiviral properties of several species of lectins, and these data have encouraged researchers to suggest lectins as a therapeutic tool against COVID-19 [57, 61]. Though, because of their high molecular weight, lectins use in clinical trials would be facing some challenging issues, such as route of administration, the bioavailability of the administered lectins, and their possible antigenic and mitogenic properties.

In relation to the physiopathology associated with COVID-19, mainly inflammatory reactions and weakness of antioxidants status of patients, other natural substances were proposed for COVID-19 management, such as thymoquinone; the main active ingredient of *Nigella sativa* extracts. *Nigella sativa* extracts and thymoquinone were widely studied for their therapeutic potentials [64]. Both thymoquinone and *Nigella sativa* extracts have interesting pharmacological properties [65], such as anti-inflammatory, anti-cancer and anti-oxidants. The use of thymoquinone by COVID-19 patients was suggested in a recent publication [66]. Route of administration and pharmaceutical presentations of thymoquinone were suggested [67, 68] that could be of interest in clinical application.

Magnesium has hundreds of biochemical properties in human physiology. Its deficiency can cause cardiovascular, neurologic and metabolic health issues, with some physiopathology aspects that are identical to some of those encountered in COVID-19 patients, such as blood hemostasis and endothelium dysfunction, inflammation and oxidative stress [69]. Those magnesium supplementation, as suggested by others [70 - 72], could help COVID-19 patients to overcome some of the physiopathology events of the disease.

CONCLUSION

So far, in the absence of an effective vaccine against SARS-Cov-2, drug management of critical cases of COVID-19 relies on several therapeutic protocols, including antibiotics, blood clotting/fibrinolysis drugs, glucocorticoids, antimalarial and some antivirals [73]. Antimalarial chloroquine and hydroxychloroquine were used in many therapeutic protocols in various countries, though their efficacy is still under debate by the scientific community despite their many effective anti-viral and other biological properties shown in laboratory experiments and their long history as antimalarial drugs [74]. Meanwhile, vitamins (such as vitamin C and B1), prebiotics and probiotics, as well as magnesium are suggested as adjunct treatments for COVID-19, while some health promoting foods such as olive oil [75] and argan oil [76, 77] could also be of high relevance for nutritional interventions for COVID-19 patients, due to their unique vitamins and antioxidants composition (polyphenols, phytosterols, vitamin E, carotenoids, oleic acid and other essential fatty acids).

ADDENDUM

Following submission of the present paper, a clinical trial

was approved by the University Hospital Ethics Committee of Fez (Morocco) to investigate the effect of camel milk consumption as an adjunct treatment in parallel to the official drug protocol approved by the Ministry of Health of Morocco to treat COVID-19 patients. The clinical trial coordinator is Pr Mourad Errasfa.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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