Proteolytic Targets for SARS-CoV-2 Spike Protein Degradation: Hope for Systemic Detoxification

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ABSTRACT

The SARS-CoV-2 spike protein is a long-lasting foreign pathogenic protein found in cells and tissues after COVID-19 respiratory illness and COVID-19 vaccination. The spike protein incites an inflammatory response and is a potent activator of nuclear factor kappa light chain enhancer of activated B cells (NF-κB). It can adversely impact any of the vital organs, such as the heart, lungs, brain, and kidneys, by inducing a severe autoimmune attack. The spike protein has been described as the causative factor for cardiovascular complications leading to increased mortality following mRNA injections. It can cause a cytokine storm that characterizes many cases of fatal COVID-19 respiratory illness. Additionally, it drives sustained inflammation in long COVID and vaccine injury syndromes in multiple tissues and organs and also by crossing the blood-brain barrier (BBB), where protective antibodies cannot act, thereby causing severe neuroinflammation through microglial activation and NF-κB. It is therefore of utmost importance to repurpose drugs and introduce natural agents, in order to provide nontoxic therapies for spike protein injury syndromes, after COVID-19 illness, vaccination, and both exposures in combination. The systemic use of proteolytic naturally derived enzymes becomes of medical interest and could offer a safe and efficacious solution for the alleviation of both spike-protein-associated symptomatology and pathology.

Introduction

The spike glycoprotein of SARS-CoV-2 is strongly implicated as the viral protein responsible for inducing the severe pathologies caused by cytokine storm associated with COVID-19.[1] The COVID-19 (mRNA) genetic vaccination-expressed spike protein is thought to be responsible for sudden deaths and/or severe side effects.[2] Potentially because of its molecular mimicry of myocyte antigens, the spike protein has been speculated to be the cause of autoimmune-associated myocarditis resulting in death.[2] It might also lead to the impairment of innate immunity that is associated with autoimmunity and cancer.[3]

The spike protein's associated pathologies are primarily produced by inducing a severe NF-κB response in various organs,[4] which initiates a chronic inflammatory state.[5]

Amino acid sequences coded for by the injected mRNA vaccines have been found circulating in the blood 28 days post-injection.[6] Furthermore, markedly elevated levels of the full-length spike protein (33.9±22.4 pg/mL), unbound by antibodies, were detected in the plasma of individuals with postvaccine myocarditis, whereas no free spike was detected in asymptomatic vaccinated control subjects.[7] This suggests that therapy that can successfully remove the protein from the circulation might be beneficial.

A high concentration of circulating full-length spike protein in plasma causes haemagglutination, and this is related to high incidences of morbidity.[8] Usually, in patients with mild COVID-19, the spike protein is cleared from the blood even before 15 days post-infection by specific T cell and antibody responses. By contrast, the persistence of spike protein circulating in the blood is encountered only in COVID-19 patients with severe symptoms requiring oxygen supplementation.[9] The presence of spike S1 protein in CD16+ monocytes up to 15 months after COVID-19 infection has been implicated in the pathology of long COVID,[10] and it has been hypothesized that it could be the main factor responsible for long COVID.[11]

There is an urgent need to establish a therapeutic approach that has as its focus the circulating spike protein, preventing it from initiating an inflammatory and/or autoimmune reaction in tissues it is known to target such as the heart and the central nervous system. Equally important, this therapeutic approach should, to the greatest extent possible, counteract the NF-kB activation and its related pathologies. This inflammatory activation is associated with both mRNA genetic vaccinations and long COVID, which share common symptoms. For example, long COVID has a significant association with the development of postural orthostatic tachycardia syndrome (POTS).[12] Likewise, tachycardia has been documented following mRNA genetic vaccination.[13,14] Additionally, ventricular fibrillation was described as a contributing causal factor in a myocarditis-associated death due to cardiac arrest following an mRNA genetic therapy ("vaccination").[15] Similarly, ventricular tachycardia, which can result in ventricular fibrillation, has been described in association with long COVID.[16] This overlap in the cardiac and autonomic symptoms associated with both long COVID and mRNA injection sequelae strongly suggests that they could both benefit from a common therapeutic approach.

Spike Protein Degradation

The SARS-CoV-2 spike protein monomers naturally assemble into a trimeric structure.[17] The protein is composed of two subunits, both of which contribute to its associated pathologies. The S1 subunit includes the receptor binding domain (RBD) that engages the angiotensin-converting enzyme 2 (ACE2) human receptor, and the S2 subunit facilitates cell fusion and viral entry.[18,19]

A possible therapeutic approach would rely on the use of natural agents that can possibly result in proteolytic cleavage and hence change the tertiary structure of circulating spike protein in the blood before it is able to interact with ACE2 receptors. The same proteolytic agents could render impaired the spike proteins expressed on the antigen-presenting immune cells following an mRNA vaccination. Such a spike

protein inactivation would also mitigate its NF-κB-induced pathology.[4,5] Proteolytic enzymes that degrade the spike protein have been recently described,[20] and they take on significant therapeutic import as a means of combatting spike-protein-induced coagulopathies, preventing the associated inflammation, and potentially mitigating other spike-protein-induced neurodegenerative effects.

Nattokinase (NK)

One candidate agent is nattokinase (NK), a fibrinolytic enzyme found in the vegetable cheese natto, which is a typical soybean food eaten in Japan.[21] NK is produced by Bacillus subtilis var. natto and is known for its traditional medicinal properties. It has been a common component of the Japanese diet for centuries. NK is a serine protease, which efficiently degrades fibrins and plasmin substrates.[21,22] Oba et al. found that a natto extract contains a protease(s) that inhibits viral infection, such as by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as well as bovine herpesvirus 1 (BHV-1), through the proteolysis of the viral proteins.[22] Tanikawa et al.[23] found that NK degraded the spike protein of SARS-CoV-2 in a dose-dependent manner and that it also degraded the spike protein on the cell surface. In addition, in this study the NK was found to efficiently degrade the RBD of the SARS-CoV-2 spike protein. Furthermore, in HEK293 transfected cells, the NK was shown to degrade the expressed spike protein on the surface of these cells, without conferring any cytotoxicity. The authors of the study stated that NK can be used as a protective agent for cells.

Natto consumption has been linked to reduced mortality from cardiovascular disease, possibly explained by its proteolytic effects in addition to its anticoagulant and antiplatelet activities.[24] However, as industrial-scale production of NK using E. coli is becoming a reality, it is important to point out some potential detrimental aspects of its therapeutic utilization.[25] NK's proteolytic activity is not specific for the spike protein and can also degrade other essential proteins of the human organism such as the housekeeping protein glyceraldehyde-3-phosphate dehydrogenase (GAPDH), although this effect had no apparent impact on cell viability.[16,23] Moreover, NK can be an allergen, and certain individuals acquire atopic dermatitis from its consumption.[26] Moreover, use in the context of concurrent pharmaceutical anticoagulants is contraindicated without close supervision of clotting parameters.

Alkaline Serine Protease

Very recently, another serine protease, this time with greater sensitivity to its spike protein substrate, has been described as a valuable naturally derived therapeutic agent against SARS-CoV-2.[20] This is the alkaline serine protease (ASP) with acidic isoelectric point (pI), known as ASPNJ. The NJ stands for the Neanthes jabonika worm from which the ASP is being extracted and purified. The ASPNJ specificity relies on the enzyme's efficiency to cleave asparagine (R) and lysine (K) amino-acid-rich regions near the carbonyl group of peptide sequences. ASPNJ has strong peptidolytic activity in its affinity for thrombin compared to the S-2238 chromogenic substrate commonly used in thrombin and prothrombin assays.[27] Thus, ASPNJ is also used for the determination of levels of prothrombin, heparin, and antithrombin in plasma.[28] The fibrinolytic and proteolytic properties of ASPNJ have been found to be useful against acute promyelocytic leukaemia cells in vitro by increasing their sensitivity to the chemotherapy drugs doxorubicin and cytarabine, whilst showing negligible inhibitory effect on normal neutrophil cells.[29]

Liu et al.[20] used ASPNJ to degrade the SARS-CoV-2 spike protein in a highly efficient manner. It was found that this serine protease digested both the full-length spike protein as well as the S1 subunit and the RBD of the SARS-CoV-2 variants. As stated by the authors, since the pI of ASPNJ is 4.4, and the pI of the RBD, S1, and S (the complete spike protein) are 8.91, 8.24, and 6.24, respectively, this low pI can lead to enhancement of the binding affinity between the enzyme and either fragments of the spike protein or the whole spike protein through the strong electrostatic interaction. It is also feasible to suggest that the alkaline enzyme can work as a scavenger molecule clearing up the spike protein from the bloodstream.

Additionally, since these electrostatic interactions are even stronger in the SARS-CoV-2 new variants, the use of ASPNJ can prove to be even more useful in the management of new variant infections.[30]

Moreover, the ASPNJ has been used in *in vivo* experiments which showed that it has no toxicity in organs such as the liver, the heart, and the kidneys of animals, while demonstrating a significant reduction of reperfusion injuries. In this in vivo model, the animals were genetically suffering from a middle artery cerebral occlusion making them susceptible to acute ischemic stroke.[31] Given the high risk of life-threatening thrombotic incidents following the mRNA genetic therapy vaccination,[32] the anticoagulant properties of the ASPNJ enzyme[29,32] have the potential to prove extremely useful for therapy in humans. Therefore, relevant clinical studies are urgently needed to establish further the safety and efficacy of ASPNJ for both the prevention and treatment of cardiovascular inflammation and injuries induced by the mRNA injections and also documented in long COVID syndrome.

The Fibrinolytic and Caseinolytic Serratiopeptidase

Serratiopeptidase (SEPD), also referred to in the literature as Serratia E-15 protease, serrapeptase, and serratioprotease, is an enzyme with several well-established clinical applications, including through its anti-inflammatory, fibrinolytic, mucolytic, and other properties.[33,34] Both clinical and preclinical studies on SEPD have established its safety profile, including as a mucolytic and anti-inflammatory agent in the treatment of COVID-19-associated respiratory illness.[33]

Of its multiple pharmacological effects, the fibrinolytic/caseinolytic properties of SEPD can prove important in counteracting the mRNA injection sequelae and in treating long COVID syndrome.[35] However, this remains to be experimentally proved by clinical studies. SEPD's mucolytic properties[36] can enhance the innate immune defense of the respiratory tract, which could be of significant benefit in the treatment of long COVID patients.[37]

Furthermore, the anti-inflammatory effects of SEPD are shown in *in vitro* experiments. Although this evidence concerns microbial (Staph. aureus) evasion of cultured osteoclasts, the

involvement of monocyte chemotactic protein (MCP-1) and therefore inhibition of interleukin-6 (IL-6) production can prove highly relevant with respect to the inflammatory side-effects related to the spike protein.[38,39] IL-6 plays a central role in the induction of severe inflammation by the NF-κB response that leads to vascular damage.[40] The spike protein strongly induces NF-κB-associated inflammation by activating monocytes and macrophages.[41] The NF-κB signal is relayed to lymphocytes, epithelial cells, and other tissue monocytes and macrophages via chemokines, and, concurrently, further triggers a cascade of Interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and IL-6 expression, fully reviewed by Turner et al.[42]. This inflammatory cascade can be transmitted throughout both the cardiovascular system and the brain by activated microglial cells, which are also induced to produce a potent NF-κB response by the spike protein.[43] Thus, the control of the NF-κB inflammatory signalling, which is produced by the circulating spike protein and which also crosses the BBB, is a primary therapeutic goal in mRNA vaccinees and long-COVID patients. Moreover, it has been shown that a substantial population of monocytes (the non-classical CD16+ monocytes) carry fragments of the spike protein for several months following SARS-CoV-2 infection.[10]

Post-mortem evidence shows that the spike protein can be found infiltrating the heart tissue of individuals who died within 20 days following an mRNA injection, demonstrating that this short time period is sufficient for spike protein accumulation in cardiac tissue following mRNA injection; that it can trigger acute (epi-) myocarditis; and that it is reported as playing a causal role in those associated deaths.[1] We suggest that the combined anti-inflammatory and anticoagulant effects of SEPD[37] could prove lifesaving for spike-protein-harmed patients, whether the harm is initiated by mRNA injection or natural infection.

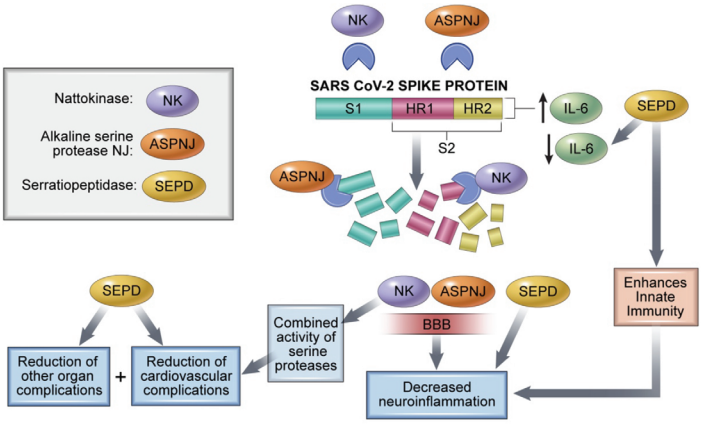

Figure 1. Potential Therapeutic Activity of Proteolytic Enzyme Administration

Finally, SEPD and NK have been shown to actively downregulate the IL-6 inflammatory signalling in brain tissue and mitigate multiple pathological changes characteristic of Alzheimer disease (AD). These enzymes have been suggested as therapeutic agents to mitigate the progression and outcome of this devastating disease.[47] Recently, our team has documented the spike protein's central role in promoting multiple pathways of neurodegenerative disease pathology.[45] It is reasonable to suggest that the therapeutic application of proteolytic enzymes could prevent and/or successfully treat these neurological sequelae of spike protein exposure.

Figure 1 presents the combined mechanisms of proteolytic enzymes that can potentially confer therapeutic benefits in treating long COVID and adverse reactions from mRNA injections. NK degrades the entire spike protein nonspecifically.[23] ASPNJ degrades specifically the S1 subunit and HR1 and HR2 domains of the spike protein.[20] Both NK and ASPNJ are serine proteases.[27,30] Serine proteases are known to cross the BBB.[46] SEPD can be useful in combination with the other proteolytic enzymes because it lowers the inflammation induced by IL-6 in response to the spike protein, reduces neurodegeneration, and enhances the innate immunity of patients suffering from spike protein toxicity.[47]

Conclusion

The repurposing of SEPD and introduction of NK and ASPNJ to the therapeutic management of long COVID and mRNA injection adverse reactions has great potential benefit. Therapeutic use is supported by their positive effect on multiple tissues and organs, including the cardiovascular system and the brain, two systems that are significantly impacted by spike-protein-associated pathologies. These enzymes have an excellent safety profile. Human clinical trials on NK and ASPNJ, alone, together, and in combination with SEPD, are urgently needed.

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